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Innovative Technology Verification Report

Technologies for Monitoring and Measurement of Dioxin and Dioxin-like Compounds in Soil and Sediment

Xenobiotic Detection Systems, Inc.
CALUX® by XDS

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Innovative Technology Verification Report

Xenobiotic Detection Systems, Inc. CALUX® by XDS

Prepared by

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Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's natural resources. Under the mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development (ORD) provides data and scientific support that can be used to solve environmental problems, build the scientific knowledge base needed to manage ecological resources wisely, understand how pollutants affect public health, and prevent or reduce environmental risks.

The National Exposure Research Laboratory is the Agency's center for investigation of technical and management approaches for identifying and quantifying risks to human health and the environment. Goals of the Laboratory's research program are to (1) develop and evaluate methods and technologies for characterizing and monitoring air, soil, and water; (2) support regulatory and policy decisions; and (3) provide the scientific support needed to ensure effective implementation of environmental regulations and strategies.

The EPA's Superfund Innovative Technology Evaluation (SITE) Program evaluates technologies designed for characterization and remediation of contaminated Superfund and Resource Conservation and Recovery Act (RCRA) sites. The SITE Program was created to provide reliable cost and performance data in order to speed the acceptance and use of innovative remediation, characterization, and monitoring technologies by the regulatory and user community.

Effective monitoring and measurement technologies are needed to assess the degree of contamination at a site, provide data that can be used to determine the risk to public health or the environment, and monitor the success or failure of a remediation process. One component of the EPA SITE Program, the Monitoring and Measurement Technology (MMT) Program, demonstrates and evaluates innovative technologies to meet these needs.

Candidate technologies can originate within the federal government or the private sector. Through the SITE Program, developers are given the opportunity to conduct a rigorous demonstration of their technologies under actual field conditions. By completing the demonstration and distributing the results, the agency establishes a baseline for acceptance and use of these technologies. The MMT Program is managed by the ORD's Environmental Sciences Division in Las Vegas, Nevada.

Gary Foley, Ph.D.
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Abstract

A demonstration of technologies for determining the presence of dioxin and dioxin-like compounds in soil and sediment was conducted under the U.S. Environmental Protection Agency's (EPA's) Superfund Innovative Technology Evaluation Program in Saginaw, Michigan, at Green Point Environmental Learning Center from April 26 to May 5, 2004. This innovative technology verification report describes the objectives and the results of that demonstration, and serves to verify the performance and cost of the Xenobiotic Detection Systems, Inc., CALUX® by XDS. Four other technologies were evaluated as part of this demonstration, and separate reports have been prepared for each technology. The objectives of the demonstration included evaluating the technology's accuracy, precision, sensitivity, sample throughput, tendency for matrix effects, and cost. The test also included an assessment of how well the technology's results compared to those generated by established laboratory methods using high-resolution mass spectrometry (HRMS). The demonstration objectives were accomplished by evaluating the results generated by the technology from 209 soil, sediment, and extract samples. The test samples included performance evaluation (PE) samples (i.e., contaminant concentrations were certified or the samples were spiked with known contaminants) and environmental samples collected from 10 different sampling locations.

The Xenobiotic Detection Systems, Inc., CALUX® by XDS is an aryl hydrocarbon-receptor bioassay that individually reports the total toxicity equivalents (TEQ) of dioxins/furans and polychlorinated biphenyls (PCBs) in the sample. As part of the performance evaluation, the technology results were compared to TEQ results generated by a reference laboratory, AXYS Analytical Services, using EPA Methods 1613B and 1668A. When comparing the CALUX® by XDS results with HRMS TEQ results from the certified samples and the reference methods, the reader should keep in mind the limitations of the TEQ approach, noting that it is possible that Ah-receptor binding compounds that are being measured during the CALUX® by XDS analysis are not all accounted for in the reference laboratory TEQ result and that the World Health Organization toxicity equivalency factors used to generate the reference laboratory TEQs may differ from the assay Ah-receptor binding affinity for certain analytes. Therefore, the technology should not be viewed as producing an equivalent measurement value to HRMS TEQ values for all samples. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint.

The CALUX® by XDS generally reported data higher than the certified PE and reference laboratory values for $TEQ_{D/F}$ and total TEQ, but were generally lower than the certified PE and reference laboratory values for TEQ_{PCB}. The technology's estimated method detection limit was similar to what was reported by the developer (0.53 to 0.63 pg/g $TEQ_{D/F}$). No statistically significant matrix effects were observed by matrix type (soil vs. sediment vs. extract) or polynuclear aromatic hydrocarbon concentration. Twenty-one percent of the CALUX® by XDS results from replicate sample sets that were analyzed in the laboratory and in the field showed a significant statistical difference, and only total TEQ value showed a statistically significant effect due to sample type (performance evaluation vs. environmental vs. extract). The technology had a fairly high rate of false positive and false negative results around 1 picogram/gram (pg/g) TEQ_{PCB} (15% and 23%, respectively), but it had significantly fewer false positives and false negatives for total TEQ (4% and 1%, respectively) and $TEQ_{D/F}$ (6% and 0%, respectively). When comparing XDS's results to the reference laboratory for samples above and below 50 pg/g TEQ, all of the false positive and false negative rates for all TEQ types were less than 10%. These data suggest that the XDS technology could be an effective tool to screen for samples above or below 1 pg/g TEQ for $TEQ_{D/F}$ and total TEQ, and that it could be effective for all three types of TEQ values to determine results above or below 50 pg/g TEQ, particularly considering that both the cost ($89,564 vs. $398,029) and the time (six weeks vs. eight months) to analyze the 209 demonstration samples were significantly less than that of the reference laboratory.

Contents

Contents (continued)

Contents (continued)

Contents (continued)

Page

Figures

Tables

Abbreviations, Acronyms, and Symbols

Ah	aryl hydrocarbon
ANOVA	analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
CALUX	Chemical-Activated LUciferase eXpression
CIL	Cambridge Isotope Laboratories
CoA	Certificate of Analysis
COC	chain of custody
CRM	certified reference material
DER	data evaluation report
D/F	dioxin/furan
DIPS	Dioxin/Furan and PCB-Specific
DMSO	dimethyl sulfoxide
DNR	Department of Natural Resources
D/QAPP	demonstration and quality assurance project plan
ELC	Environmental Learning Center
EMDL	estimated method detection limit
EMPC	estimated maximum possible concentration
EPA	Environmental Protection Agency
ERA	Environmental Resource Associates
FDA	Food and Drug Administration
g	gram
GC	gas chromatography
HPLC/GPC	high-performance liquid chromatography/gel permeation chromatography
HRGC	high-resolution capillary gas chromatography
HRMS	high-resolution mass spectrometry
i.d.	internal diameter
IDW	investigation-derived waste
ITVR	innovative technology verification report
kg	kilogram
L	liter
LRMS	low-resolution mass spectrometry
µm	micrometer
m	meter

Abbreviations, Acronyms, and Symbols (Continued)

MDEQ	Michigan Department of Environmental Quality
MDL	method detection limit
mg	milligram
mL	milliliter
mm	millimeter
MMT	Monitoring and Measurement Technology
MS	mass spectrometry
NERL	National Exposure Research Laboratory
ng	nanogram
NIST	National Institute for Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
ORD	Office of Research and Development
PAH	polynuclear aromatic hydrocarbons
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-p-dioxin/dibenzofuran
PCDH	polychlorinated diaromatic hydrocarbon
PCP	pentachlorophenol
PE	performance evaluation
pg	picogram
PHDH	polyhalogenated diaromatic hydrocarbon
ppb	parts per billion; nanogram/g; ng/g
ppm	parts per million; microgram/g; µg/g
ppt	parts per trillion; picogram/g; pg/g
psi	pound per square inch
QA/QC	quality assurance/quality control
RM	reference material
RPD	relative percent difference
RSD	relative standard deviation
SDL	sample-specific detection limit
SIM	selected ion monitoring
SITE	Superfund Innovative Technology Evaluation
SOP	standard operating procedure
SRM	Standard Reference Material®
TCDD	tetrachlorodibenzo-*p*-dioxin
TEF	toxicity equivalency factor
TEQ	toxicity equivalent
$TEQ_{D/F}$	total toxicity equivalents of dioxins/furans
TEQ_{PCB}	total toxicity equivalents of World Health Organization dioxin-like polychlorinated biphenyls

Abbreviations, Acronyms, and Symbols (Continued)

TOC	total organic carbon
total TEQ	total toxicity equivalents including the sum of the dioxin/furan and World Health Organization dioxin-like polychlorinated biphenyls
WHO	World Health Organization
X-CARB	proprietary carbon matrix developed by Xenobiotic Detection Systems, Inc.
XDS	Xenobiotic Detection Systems, Inc.

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Chapter 1
Introduction

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Exposure Research Laboratory (NERL) contracted with Battelle (Columbus, Ohio) to conduct a demonstration of monitoring and measurement technologies for dioxin and dioxin-like compounds in soil and sediment. A field demonstration was conducted as part of the EPA Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program. The purpose of this demonstration was to obtain reliable performance and cost data on the technologies to provide (1) potential users with a better understanding of the technologies' performance and operating costs under well-defined field conditions and (2) the technology developers with documented results that will help promote the acceptance and use of their technologies.

This innovative technology verification report (ITVR) describes the SITE MMT Program and the scope of this demonstration (Chapter 1); the Xenobiotic Detection Systems, Inc. (XDS), CALUX® (Chemical-Activated LUciferase eXpression) by XDS (Chapter 2); the demonstration site and the sampling locations (Chapter 3); the demonstration approach (Chapter 4); the confirmatory process (Chapter 5); the assessment of reference method data quality (Chapter 6); the performance of the technology (Chapter 7); the economic analysis for the technology and reference method (Chapter 8); the demonstration results in summary form (Chapter 9); and the references used to prepare this report (Chapter 10). Appendix A contains a verification statement; Appendix B contains supplemental information provided by the developer; Appendix C is a summary of method blank and batch duplicate data by the reference laboratory; and Appendix D contains a one-to-one matching of the developer and reference laboratory data.

1.1 Description of the SITE MMT Program

Performance verification of innovative environmental technologies is an integral part of the regulatory and research mission of the EPA. The SITE Program was established by the EPA Office of Solid Waste and Emergency Response and ORD under the Superfund Amendments and Reauthorization Act of 1986. The overall goal of the Program is to conduct performance verification studies and to promote the acceptance of innovative technologies that may be used to achieve long-term protection of human health and the environment. The program is designed to meet three primary objectives: (1) identify and remove obstacles to the development and commercial use of innovative technologies, (2) demonstrate promising technologies and gather reliable performance and cost information to support site characterization and remediation activities, and (3) develop procedures and policies that encourage use of innovative technologies at Superfund sites as well as at other waste sites or commercial facilities. The SITE Program includes the following elements:

- MMT Program—Evaluates technologies that sample, detect, monitor, or measure hazardous and toxic substances. These technologies are expected to provide better, faster, or more cost-effective methods for producing real-time data during site characterization and remediation efforts than conventional laboratory technologies.

- Remediation Technology Program—Conducts demonstrations of innovative treatment technologies to provide reliable performance, cost, and applicability data for site cleanups.

- Technology Transfer Program—Provides and disseminates technical information in the form of updates, brochures, and other publications

that promote the SITE Program and participating technologies. It also supports the technologies by offering technical assistance, training, and workshops.

The MMT Program's technology verification process is designed to conduct demonstrations that will generate high-quality data so that potential users have reliable information regarding the technology performance and cost. Four steps are inherent in the process: (1) needs identification and technology selection, (2) demonstration planning and implementation, (3) report preparation, and (4) information distribution. The first step of the technology verification process begins with identifying technology needs of the EPA and regulated community. The EPA Regional offices, the U.S. Department of Energy, the U.S. Department of Defense, industry, and state environmental regulatory agencies are asked to identify technology needs for sampling, measurement, and monitoring of environmental media. Once a need is identified, a search is conducted to identify suitable technologies that will address the need. The technology search and identification process consists of examining industry and trade publications, attending related conferences, and exploring leads from technology developers and industry experts. Selection of technologies for field testing includes evaluation of the candidate technologies based on several criteria. A suitable technology for field testing

- is designed for use in the field or in a mobile laboratory,
- is applicable to a variety of environmentally contaminated sites,
- has potential for solving problems that current methods cannot satisfactorily address,
- has estimated costs that are lower than those of conventional methods,
- is likely to achieve equivalent or better results than current methods in areas such as data quality and turnaround time,
- uses techniques that are easier or safer than current methods, and
- is commercially available.

Once candidate technologies are identified, developers are asked to participate in a developer conference. This conference gives the developers an opportunity to describe their technologies' performance and to learn about the MMT Program.

The second step of the technology verification process is to plan and implement a demonstration that will generate representative, high-quality data to assist potential users in selecting a technology. Demonstration planning activities include a pre-demonstration sampling and analysis investigation that assesses existing conditions at the proposed demonstration site or sites. The objectives of the pre-demonstration investigation are to (1) confirm available information on applicable physical, chemical, and biological characteristics of contaminated media at the sites to justify selection of site areas for the demonstration; (2) provide the technology developers with an opportunity to evaluate the areas, analyze representative samples, and identify logistical requirements; (3) assess the overall logistical and quality assurance requirements for conducting the demonstration; and (4) select and provide the reference laboratory with an opportunity to identify any matrix-specific analytical problems associated with the contaminated media and to propose appropriate solutions. Information generated through the pre-demonstration investigation is used to develop the final demonstration design and to confirm the nature and source of samples that will be used in the demonstration.

Demonstration planning activities also include preparation of a demonstration plan that describes the procedures to verify the performance and cost of each technology. The demonstration plan incorporates information generated during the pre-demonstration investigation as well as input from technology developers, demonstration site representatives, and technical peer reviewers. The demonstration plan also incorporates the quality assurance (QA)/quality control (QC) elements needed to produce data of sufficient quality to document the performance and cost of each technology.

During the demonstration, each technology is evaluated independently and, when possible and appropriate, is compared to a reference technology. The performance and cost of one technology are not compared to those of another technology evaluated in the demonstration.

Rather, demonstration data are used to evaluate the individual performance, cost, advantages, limitations, and field applicability of each technology.

As part of the third step of the technology verification process, the EPA publishes a verification statement (Appendix A) and a detailed evaluation of each technology in an ITVR. To ensure its quality, the ITVR is published only after comments from the technology developer and external peer reviewers are satisfactorily addressed. All demonstration data used to evaluate each technology are summarized in a data evaluation report (DER) that constitutes a complete record of the demonstration. The DER includes audit reports, observer reports, completed data validation checklists, certificates of analysis, and the data packages (i.e., raw data) from the reference laboratory. The DER is not published as an EPA document, but a copy may be obtained from the EPA project manager.

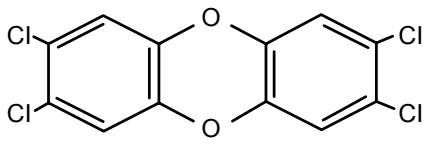
The fourth step of the verification process is to distribute demonstration information. To benefit technology developers and potential technology users, the EPA makes presentations, publishes and distributes fact sheets, newsletters, bulletins, and ITVRs through direct mailings and on the Internet. Information on the SITE Program is available on the EPA ORD Web site (http://www.epa.gov/ORD/SITE). Additionally, a Visitor's Day, which is held in conjunction with the demonstration, allows the developers to showcase their technologies and gives potential users the opportunity to have a firsthand look at the technologies in operation.

1.2 Scope of This Demonstration

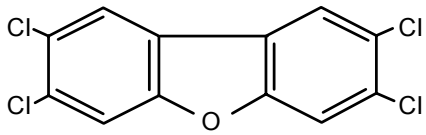
Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, commonly referred to collectively as "dioxins," are of significant concern in site remediation projects and human health assessments because they are highly toxic. Dioxins and furans are halogenated aromatic hydrocarbons and are similar in structure as shown in Figure 1-1. They have similar chemical and physical properties. Chlorinated dioxins and furans are technically referred to as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). For the purposes of this document, they will be referred to simply as "dioxins," "PCDD/F," or "D/F." Dioxins and furans are not intentionally produced in most chemical processes. However, they can be synthesized directly and are commonly generated as by-products of various combustion and chemical processes.[1] They are colorless crystals or solids with high melting points, very low water solubility, high fat solubility, and low volatility. Dioxins and furans are extremely stable under most environmental conditions, making them persistent once released in the environment. Because they are fat soluble, they also tend to bioaccumulate.

There are 75 individual chlorinated dioxins and 135 individual chlorinated furans. Each individual dioxin and furan is referred to as a congener. The properties of each congener vary according to the number of chlorine atoms present and the position where the chlorines are attached. The congeners with chlorines attached at a minimum in the 2, 3, 7, and 8 positions are considered most toxic. A total of seven dioxin and 10 furan congeners contain chlorines in the 2, 3, 7, 8 positions and, of these, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is one of the most toxic and serves as the marker compound for this class.

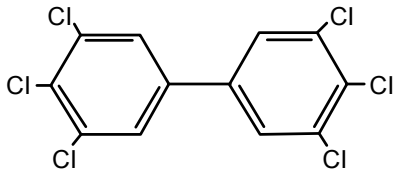
Certain polychlorinated biphenyls (PCBs) have structural and conformational similarities to dioxin compounds (Figure 1-1) and are therefore expected to exhibit toxicological similarities to dioxins as well. Currently only 12 of the total 209 PCB congeners are



2,3,7,8-Tetrachlorodibenzo-p-dioxin



2,3,7,8-Tetrachlorodibenzofuran



3,3',4,4',5,5'-Hexachlorobiphenyl

Figure 1-1. Representative dioxin, furan, and polychlorinated biphenyl structure.

thought to have "dioxin-like" toxicity. These 12 are PCBs with four or more chlorines with just one or no substitution in the ortho position, and which assume a flat configuration with rings in the same plane. These "dioxin-like" PCBs are often refered to as non-ortho and mono-ortho substituted coplanar PCBs.

Conventional analytical methods for determining concentrations of dioxin and dioxin-like compounds are time-consuming and costly. For example, EPA standard methods require solvent extraction of the sample, processing the extract through multiple cleanup columns, and analyzing the cleaned fraction by gas chromatography (GC)/high-resolution mass spectrometry (HRMS). The use of a simple, rapid, cost-effective analytical method would allow field personnel to quickly assess the extent of contamination at a site and could be used to direct or monitor remediation or risk assessment activities. This data could be used to provide immediate feedback on potential health risks associated with the site and permit the development of a more focused and cost-effective sampling strategy. At this time, more affordable and quicker analytical techniques will not replace HRMS. However, before adopting an alternative to traditional laboratory-based methods, a thorough assessment of how commercially available technologies compare to conventional laboratory-based analytical methods using certified, spiked, and environmental samples is warranted. A summary of the demonstration activities to evaluate measurement technologies for dioxin and dioxin-like compounds in soil and sediment is provided below. The experimental design and demonstration approach are described in greater detail in Chapter 4 and was published in the Demonstration and Quality Assurance Project Plan (D/QAPP).[2]

1.2.1 Organization of Demonstration

The key organizations and personnel involved in the demonstration, including the roles and responsibilities of each, are fully described in the D/QAPP.[2] EPA/NERL had overall responsibility for this project. The EPA reviewed and concurred with all project deliverables including the D/QAPP and the ITVRs, provided oversight during the demonstration, and participated in the Visitor's Day. Battelle served as the verification testing organization for EPA/NERL. Battelle's responsibilities included developing and implementing all elements of the D/QAPP; scheduling and coordinating the activities of all demonstration participants; coordinating the collection of environmental samples; serving as the characterization laboratory by performing the homogenization of the environmental samples and confirming the efficacy of the homogenization and approximate sample concentrations; conducting the demonstration by implementing the D/QAPP; summarizing, evaluating, interpreting, and documenting demonstration data for inclusion in this report; and preparing draft and final versions of each developer's ITVR. The developers were five companies who submitted technologies for evaluation during this demonstration. The responsibilities of the developers included providing input to, reviewing, and concurring with the D/QAPP; providing personnel and supplies as needed for the demonstration; operating their technology during the demonstration; and reviewing and commenting on their technologies' ITVRs. AXYS Analytical Services, Ltd. was selected to serve as the reference analytical laboratory. AXYS analyzed each demonstration sample by EPA Method 1613B[3] and EPA Method 1668A[4] according to the statement of work provided in the D/QAPP.

The Michigan Department of Environmental Quality (MDEQ) hosted the demonstration, coordinated the activities of and participated in Visitor's Day, and collected and provided some of the environmental samples that were used in the demonstration. The Dioxin SITE Demonstration Panel served as technical advisors and observers of the demonstration activities. Panel membership, which is outlined in the D/QAPP, included representation from EPA Regions 1, 2, 3, 4, 5, 7, and 9; EPA Program Offices; the MDEQ; and the U.S. Fish and Wildlife Services. Members of the panel participated in five conference calls with the EPA, Battelle, AXYS, and the developers. The panel contributed to the experimental design and D/QAPP development; logistics for the demonstration, including site selection, sample collection, reference laboratory selection, and data analysis; and technology evaluation procedures. As an example of the significant impact the panel had on the demonstration, it was the EPA members of the panel who suggested expanding the scope of the project from focusing exclusively on dioxins and furans, to also include PCBs and the generation of characterization data for polynuclear aromatic hydrocarbons (PAHs).

1.2.2 Sample Descriptions and Experimental Design

Soil and sediment samples with a variety of distinguishing characteristics such as high levels of PCBs and PAHs were analyzed by each participant. Samples were collected from a variety of dioxin-contaminated soil and sediment sampling locations around the country. Samples were identified and supplied through EPA Regions 2, 3, 4, 5, and 7 and the MDEQ. The samples were homogenized and characterized by the characterization laboratory prior to use in the demonstration to ensure a variety of homogeneous, environmentally derived samples with concentrations over a large dynamic range (< 50 to > 10,000 picogram/gram [pg/g]) were included. The environmental samples comprised 128 of the 209 samples included in the demonstration (61%). Performance evaluation (PE) samples were obtained from five commercial sources. PE samples consisted of known quantities of dioxin and dioxin-like compounds. Fifty-eight of the 209 demonstration samples (28%) were PE samples. A suite of solvent extracts was included in the demonstration to minimize the impact of sample homogenization and to provide a uniform matrix for evaluation. A total of 23 extracts (11% of the total number of samples) was included in the demonstration. The demonstration samples are described in greater detail in Section 4.3.

1.2.3 Overview of Field Demonstration

All technology developers participated in a pre-demonstration study where a representative subset of the demonstration samples was analyzed. The pre-demonstration results indicated that the XDS technology was suitable for participation in the demonstration. The demonstration of technologies for the measurement of dioxin and dioxin-like compounds was conducted at the Green Point Environmental Learning Center (ELC) in Saginaw, Michigan, from April 26 to May 5, 2004. Five technologies, including immunoassay test kits and aryl hydrocarbon (Ah)-receptor binding technologies, participated in the demonstration. The operating procedures for the participating technologies are described in the D/QAPP.

The technologies were operated by the developers. Because the sample throughput of the technologies varied widely, it was at the discretion of the developers how many of the 209 demonstration samples were analyzed in the field. Results from the demonstration samples, in comparison with results generated by AXYS using standard analytical methods, were used to evaluate the analytical performance of the technologies, including the parameters of accuracy, precision, and comparability. Observations from the field demonstration were used to assess sample throughput, ease of use, health and safety aspects, and the field portability of each technology. The performance evaluation of the CALUX® by XDS is presented in this ITVR. Separate ITVRs have been published for the other four participating technologies.

Chapter 2
Description of Xenobiotic Detection Systems, Inc., CALUX® by XDS

This technology description is based on information provided by XDS and only editorial changes were made to ensure document consistency. Actual cost and performance data, as reported and observed during the demonstration, will be provided later in this document. CALUX® by XDS technology is based on a reporter gene system using a genetically engineered cell line capable of detecting all of the WHO-recognized dioxins, furans, and PCBs. Giving results for dioxins/furans and PCBs separately or together, as well as being available as a screening and/or quantitative analysis, CALUX® by XDS is used to analyze soil, sediment, fly ash, stack gas emissions, food, feed, blood, and water suspected of being contaminated with dioxins/furans and PCBs.

2.1 Company History

XDS was started in 1995 by Drs. George C. Clark and Michael S. Denison to develop biologically based methods for analysis of toxic compounds that are harmful to animals and humans. The primary headquarters of the company are located in the city of Durham, on the edge of North Carolina's Research Triangle Park.

The CALUX® by XDS technology was first used commercially in 1996 to test milk. Its effectiveness became known internationally throughout the scientific community after its much-publicized successes in the United States. The Hiyoshi Corporation of Japan became the first licensee of XDS technology in 2000. Years of extensive burning of refuse that would normally go into landfills in Japan has resulted in extensive low-level dioxin contamination. The CALUX® by XDS technology provided Hiyoshi a cost-effective method for extensive screening of large areas of land.

In August of 2001, the Food and Drug Administration (FDA) Center for Veterinary Medicine and the FDA Office of Regulatory Affairs, Arkansas Regional Laboratory, signed a licensing agreement to use the CALUX® by XDS bioassay for investigation as a new technology in the detection of dioxin-like compounds.

XDS was selected by the Belgium government in September of 2000 to help protect the country's residents and food supply from chemical contamination. The Scientific Institute of Public Health of Belgium signed a five-year licensing agreement after XDS won a Belgium-sponsored competition that included technology entries from six other companies. Also in 2002, BELTEST (the Belgium Government's accreditation service) certified the XDS-patented bioassay technology as a valid and accurate method for screening detection of chlorinated dioxins and PCBs. As a result of this certification, XDS's patented technology is an accepted method throughout the European Union for screening dioxins and PCBs in foodstuffs. In December 2003, Prince Agri Products, Inc. of Quincy, Illinois, selected and recommended XDS to its raw material suppliers as a preferred dioxin analysis laboratory. Prince Agri Products, a leader in the trace mineral industry, manufactures and processes more trace mineral supplements than any other supplier for the animal feed industry.

Currently, XDS is preparing to market an endocrine disruptor detection bioassay. This is a cell-based transcriptional method to evaluate the endocrine disruptor activity of chemicals for the estrogen receptor. XDS has termed this test method the LUMI-CELL™ ER bioassay and has developed a standardized test

procedure in a stably transfected recombinant cell line that is sensitive, robust, and reproducible in detecting estrogen-active chemicals.

The association of exposure to endocrine (hormone) disruptor chemicals (EDCs) and adverse health effects in human and wildlife populations has led to worldwide concern. Some of the health effects that have led to this concern include global increases in testicular cancer, regional declines in sperm counts, altered sex ratios in wildlife populations, increases in the incidence of breast cancer and endometriosis, and accelerated puberty in females that are expected to result from exposure to chemicals that adversely affect steroid hormone action.

The LUMI-CELL™ ER bioassay is an extremely rapid in vitro method that can evaluate the estrogenic activity of chemicals within two days. The method also provides relative activity of a chemical to the standard beta-estradiol and provides dose response activity of the chemical. The standardized protocol developed allows for a very robust system with low variability and high sensitivity. The cost of the LUMI-CELL™ ER bioassay is a few hundred dollars per chemical, which is substantially less than any animal base method. The LUMI-CELL™ ER bioassay is a transcriptionally based assay capable of testing for antagonistic responses of EDCs, which is not possible using other binding assays.

2.2 Product History

In 1998, XDS was awarded a patent (U.S. patent number 5,854,010) for its proprietary CALUX® by XDS assay for dioxin-like chemicals. XDS genetically engineered mammalian cell lines to contain the gene for luciferase, an enzyme fireflies use to produce light. In the patented CALUX® by XDS process, firefly luciferase is produced when dioxin-like chemicals are present. The amount of light produced is directly related to the amount of dioxin-like chemicals. The process detects dioxin at levels below one part per trillion, and costs 40% to 70% less than traditional high-resolution GC/HRMS.

In April 2004, XDS was awarded a second U.S. patent (U.S. patent number 6,720,431 B2), further improving the CALUX® by XDS bioassay. This certification was regarding a method for separating the polyhalogenated diaromatic hydrocarbon (PHDH) toxicity equivalents (TEQs) of the PCDD/F subgroup from the TEQs of the PCB compounds and reporting these results separately. This new method is a major step forward in toxin detection as it allows for multiple analysis results from one PHDH laboratory sample. This saves time and is extremely cost efficient for both research and general public applications. The new process also provides a method for eliminating compounds that are not of the PHDH chemical group. This process provides nearly identical savings to the first patented process.

Further development of the CALUX® by XDS technology was supported by Small Business Innovation Research grants (1R43 ES08327-01 and 2R44 ES08372-02) from the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, one of the National Institutes of Health.

2.3 Technology Description

XDS has patented (U.S. patent number 5,854,010) a genetically engineered cell line that contains the firefly luciferase gene under transactivational control of the Ah receptor. This cell line can be used for the detection and quantification of the Ah-receptor agonists, the target receptor of dioxins, furans, and PCBs. The XDS term for the *in vitro* assay is the CALUX® by XDS assay. The most widely studied compounds that activate this system are the polychlorinated diaromatic hydrocarbons (PCDH), such as 2,3,7,8-TCDD. Many PCDH compounds are quantified relative to TCDD, since this is one of the most potent activators of Ah-receptor mediated gene transcription. These relative quantifications are known as TEQs, and the results from the CALUX® by XDS assay provide a measure of TEQs in a sample. By using patented cleanup methods developed by XDS, it is possible to separate PCBs from dioxins/dibenzofurans and to determine what portion of the total TEQ in a sample is due to each of these classes of compounds. XDS has termed this procedure the Dioxin/Furan and PCB-Specific (DIPS) or DIPS-CALUX® by XDS bioassay.

Prices start at $200 for a dioxin screening (single) analysis and $250 for a dioxin and PCB analysis, with analysis provided as a fee for service at the XDS laboratories. Field analysis is available with 96-well

plates being shipped to the site for analytical procedures to be performed by trained personnel. Costs per 96-well plates are approximately $2,400, with each plate capable of analyzing up to 40 samples along with standard curves and quality control standards. Rental of equipment and proprietary software to perform the CALUX® by XDS is also available.

The CALUX® by XDS bioassay for dioxin-like chemicals uses a patented sample processing procedure (U.S. patent number 6,720,431) that allows separation of coplanar PCBs and PCDDs/PCDFs so that estimates of TEQ can be made for each chemical class. This allows reporting of TEQ estimates for chlorinated dioxins/furans and for the PCBs. The samples are extracted using a modification of the EPA SW-846 Method 8290 extraction method. Briefly, the dried samples are ground, and 1-g aliquots are placed in solvent-cleaned glass vials with polytetrafluoroethylene-lined caps. The sample is extracted with a 20% solution of methanol in toluene and then twice with toluene. During each extraction step, the samples are sonicated in an ultrasonic water bath. The three extracts from each sample are filtered, pooled, and concentrated by vacuum centrifugation. The sample extract is suspended in hexane and rapidly processed through a patented two-column chromatographic procedure to produce two extracts, one containing chlorinated dioxins/furans and one containing PCBs (see Figure 2-1). The extracts are exchanged into dimethyl sulfoxide (DMSO) and used to dose the genetically engineered cells in the CALUX® assay by XDS to provide TEQ estimates for PCBs and PCDD/PCDFs. Prior to dosing the cells, the sample extracts in DMSO are suspended in cell culture medium. This medium is then used to expose monolayers of the H1L1 cell line grown in 96-well culture plates (see Figure 2-2). In addition to the samples, a standard curve of

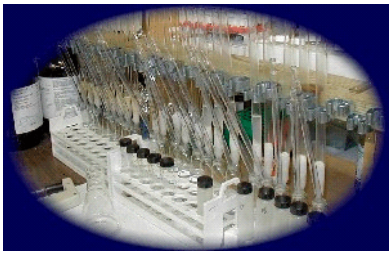


Figure 2-1. XDS patented sample processing procedure.

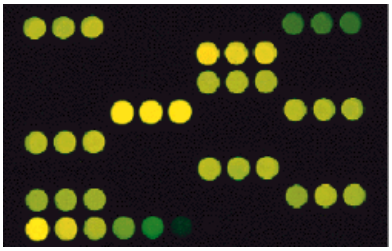


Figure 2-2. Luminescence produced when CALUX® by XDS cells are exposed to dioxin and dioxin-like chemicals.

2,3,7,8-TCDD is assayed [250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98, 0.49, and 0.24 parts per trillion (ppt) TCDD]. The plates are incubated for a time to produce optimal expression of the luciferase activity in a humidified CO_2 incubator. Following incubation, the medium is removed and the cells are examined microscopically for viability. The induction of luciferase activity is quantified using the luciferase assay kit from Promega.

This is the developer method that was implemented during the field demonstration. A photo of the technology in operation during the demonstration is presented in Figure 2-3. XDS provided supplemental information about the performance of their technology during the demonstration and it is presented in Appendix B.

Figure 2-3. XDS processing samples during the field demonstration.

2.4 Developer Contact Information

Additional information about this technology can be obtained by contacting:

Xenobiotic Detection Systems, Inc.
Dr. John Gordon
1601 E. Geer Street, Suite S
Durham, North Carolina 27704
Telephone: (919) 688-4804
E-mail: johngordon@dioxins.com
Website: www.dioxins.com

Information was provided by the developer and does not necessarily reflect the opinion of the EPA.

Chapter 3
Demonstration and Environmental Site Descriptions

This chapter describes the demonstration site, the sampling locations, and why each was selected.

3.1 Demonstration Site Description and Selection Process

This section describes the site selected for hosting the demonstration, along with the selection rationale and criteria. Several candidate host sites were considered. The candidate sites were required to meet certain selection criteria, including necessary approvals, support, and access to the demonstration site; enough space and power to host the technology developers, the technical support team, and other participants; and various levels of dioxin-contaminated soil and/or sediment that could be analyzed as part of the demonstration. Historically, these demonstrations are conducted at sites known to be contaminated with the analytes of interest. The visibility afforded the sites is a valuable way of keeping the local community informed of new technologies and to help promote the EPA's commitment to promote and advance science and communication.

After review of the information available, the site selected for the demonstration was the Green Point ELC site, located within the city of Saginaw, Michigan. The Saginaw city-owned, 76-acre Green Point ELC, formerly known as the Green Point Nature Center, is managed by the Shiawassee National Wildlife Refuge. The Green Point ELC is situated within the Tittabawassee River flood plain. The MDEQ found higher than normal levels of dioxins in soil and sediment samples taken from the Tittabawassee River. The flood plain is not heavily laden with PCBs; however, low levels of PCBs have been detected in some areas. Soil samples taken from areas outside the flood plain were at typical background levels. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing.

To summarize, Green Point ELC was selected as the demonstration site based on the following criteria:

- Access and Cooperation of the State and Local Community—Representatives from the MDEQ, EPA Region 5, and the local U.S. Fish and Wildlife Services supported the demonstration by providing site access for the demonstration, logistical support for the demonstration, and supported a Visitor's Day during the demonstration.

- Space Requirements and Feasibility—The demonstration took place in the parking lot adjacent to the Green Point ELC, not directly on an area of contamination. The site had electrical power and adequate space to house the trailers and mobile labs that were used for the demonstration. Furthermore, the site was close to an international airport. The weather in Michigan at the time of the demonstration was unpredictable; however, all participants were provided heated containment (a mobile laboratory or construction trailer).

- Site Diversity—The area encompassing the Green Point site had different levels and types of dioxin contamination in both the soil and sediment that were used to evaluate the performance of the technologies.

The demonstration was conducted at the Green Point ELC over a 10-day period from April 26 to May 5, 2004. All technologies were operated inside trailers equipped with fume hoods or inside mobile laboratories. As such, the ambient weather conditions during the demonstration had little impact on the operation of the technologies,

since all of the work spaces were climate-controlled with heat and air conditioning. The outdoor weather conditions were generally cool and rainy, but the developers kept their working environment at comfortable temperatures (16 to 18°C). The low temperature over the 10-day demonstration period was 2°C, the high temperature was 26°C, and the average temperature was 9°C. Precipitation fell on eight of the 10 days, usually in the form of rain, but occasionally as sleet or snow flurries, depending on the temperature. The largest amount of precipitation on a given demonstration day was 0.50 inches.

3.2 Description of Sampling Locations

This section provides an overview of the 10 sampling sites and methods of selection. Table 3-1 summarizes each of the locations, what type of sample (soil or sediment) was provided, the number of samples submitted from each location, and the number of samples included in the demonstration from each location. Samples were collected from multiple sampling sites so that a wide variety of matrix conditions could be used to evaluate the performance of the technologies in addressing monitoring needs at a diverse range of Superfund sites.

Samples consisted of either soil or sediment and are described below based on this distinction. It should be noted that it was not an objective of the demonstration to accurately characterize the concentration of dioxins, furans, and PCBs from a specific sampling site. It was, however, an objective to ensure comparability between technology samples and the reference laboratory samples. This was accomplished by homogenizing each matrix, such that all sub-samples of a given matrix had consistent contaminant concentrations. As a result, homogenized samples were not necessarily representative of original concentrations at the site.

3.2.1 Soil Sampling Locations

This section provides descriptions of each of the soil sampling locations, including how the sites became contaminated and approximate dioxin concentrations, as well as the type and concentrations of other major constituents, where known [such as PCBs, pentachlorophenol (PCP), and PAHs]. This information was provided by the site owners/sample providers (e.g., the EPA, EPA contractors, and the MDEQ).

3.2.1.1 Warren County, North Carolina

Five areas of the Warren County PCB Landfill in North Carolina, a site with both PCB and dioxin contamination, were sampled. Dioxin concentrations in the landfill soils range approximately from 475 to 700 pg/g, and PCB concentrations are greater than 100 parts per million (ppm). The Warren County PCB Landfill contains soil that was contaminated by the illegal spraying of waste transformer oil containing PCBs from over 210 miles of highway shoulders. Over 30,000 gallons of contaminated oil were disposed of in 14 North Carolina counties. The landfill is located on a 142-acre tract of land. The EPA permitted the landfill under the Toxic Substances Control Act. Between September and November 1982, approximately 40,000 cubic yards (equivalent to 60,000 tons) of PCB-contaminated soil were removed and hauled to the newly constructed landfill located in Warren County, North Carolina. The landfill is equipped with both polyvinyl chloride and clay caps and liners. It also has a dual leachate collection system. The material in the landfill is solely from the contaminated roadsides. The landfill was never operated as a commercial facility. The remedial action was funded by the EPA and the State of North Carolina. The site was deleted from the National Priorities List on March 7, 1986.

3.2.1.2 Tittabawassee River Flood Plain

The MDEQ sampled the Tittabawassee River flood plain soils from three sites in the flood plain. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing. Two samples were collected from two locations at Imerman Park in Saginaw Township. The first sample was taken near the boat launch, and the second sample was taken in a grassy area near the river bank. Previous analysis from these areas of this park indicated a range of PCDD/F concentrations from 600 to 2,500 pg/g. Total PCBs from these previous measurements were in the low ppt range. Two samples were collected from two locations at Freeland Festival Park in Freeland, MI. The first sample was taken above the river bank, and the second sample was taken near a brushy forested area within the park complex. Previous PCDD/F concentrations were from 300 to 3,400 pg/g, and total PCBs were in the low ppt range. The final two samples were collected from Department of Natural Resources (DNR)-owned property in Saginaw, which was formerly a farming area

Table 3-1. Summary of Environmental Sampling Locations

Sample Type	Sampling Location	Number of Samples	
		Submitted for Consideration	Included in Demonstration
Soil	Warren County, North Carolina	5	3
	Tittabawassee River, Michigan	6	3
	Midland, Michigan	6	4
	Winona Post, Missouri	6	3
	Solutia, West Virginia	6	3
Sediment	Newark Bay, New Jersey	6	4
	Raritan Bay, New Jersey	6	3
	Tittabawassee River, Michigan	6	3
	Saginaw River, Michigan	6	3
	Brunswick, Georgia	5	3
Total		***58***	***32***

located almost at the end of the Tittabawassee River where it meets the Shiawassee River to form the Saginaw River. Previous PCDD/F concentrations ranged from 450 to 1,150 pg/g. Total PCBs were not previously analyzed, but concentrations were expected to be less than 1 ppm. The DNR property is approximately a 10-minute walk from where the demonstration was conducted at the Green Point ELC.

3.2.1.3 Midland, Michigan

Soil samples were collected by the MDEQ from various locations in Midland, Michigan. The soil type and nature of dioxin contamination are different in the Midland residential area than it is on the Tittabawassee River flood plain, but it is from the same suspected source (legacy contamination from chemical manufacturing). Samples were collected in various locations around Midland. Estimated TEQ concentrations ranged from 10 pg/g to 1,000 pg/g.

3.2.1.4 Winona Post

The Winona Post site in Winona, Missouri, was a Superfund cleanup of a wood treatment facility. Contaminants at the site included PCP, dioxin, diesel fuel, and PAHs. Over a period of at least 40 years, these contaminants were deposited into an on-site drainage ditch and sinkhole. Areas of contaminant deposition (approximately 8,500 cubic yards of soils/sludge) were excavated in late 2001/early 2002. This material was placed into an approximate 2½-acre treatment cell located on facility property. During 2002/2003, material at the treatment cell was treated through addition of amendments (high-ammonia fertilizer and manure) and tilling. Final concentrations achieved in the treatment cell averaged 26 milligram (mg)/kilogram (kg) for PCP and from 8,000 to 10,000 for pg/g dioxin equivalents. Samples obtained for this study from this site were obtained from the treatment cell after these concentrations had been achieved.

3.2.1.5 Solutia

The chemical production facility at the Solutia site in Nitro, West Virginia, is located along the eastern bank of the Kanawha River, in Putnam County, West Virginia. The site has been used for chemical production since the early 1910s. The initial production facility was developed by the U.S. government for the production of military munitions during the World War I era between 1918 and 1921. The facility was then purchased by a small private chemical company, which began manufacturing chloride, phosphate, and phenol compounds at the site. A major chemical manufacturer purchased the facility in 1929 from Rubber Services Company. The company continued to expand operations and accelerated its growth in the 1940s. A variety of raw materials has been used at the facility over the years, including inorganic compounds, organic solvents, and other organic compounds, including Agent Orange. Agent Orange is a mixture of chemicals containing equal amounts of two herbicides: 2,4-D (2,4 dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5 trichlorophenoxyacetic acid). Manufacture of this chemical herbicide began at the site in 1948 and ceased in 1969. The source of the dioxin contamination in the

site soils was associated with the manufacture of 2,4,5-T, where dioxins are an unintentional by-product. The site has a dioxin profile from ppt to low parts per billion (ppb) range. No PCBs or PAHs were identified in the soil.

3.2.2 Sediment Sampling Sites

This section provides descriptions of each of the sediment sites that includes how the sites became contaminated and approximate dioxin concentrations, as well as the type and concentrations of other major constituents (such as PCBs, PCP, and PAHs). This information was provided from site owners/samples providers (e.g., the EPA, EPA contractors, and the MDEQ).

3.2.2.1 New York/New Jersey Harbors

Dredged materials from the New York and New Jersey harbors were provided as samples for the demonstration. The U.S. Army Corps of Engineers, New York District, and EPA Region 2 are responsible for managing dredged materials from the New York and New Jersey harbors. Dioxin levels affect the disposal options for dredged material. Dredged materials are naturally occurring bottom sediments, but some in this area have been contaminated with dioxins and other compounds by municipal or industrial wastes or by runoff from terrestrial sources such as urban areas or agricultural lands.

3.2.2.1.1 Newark Bay

Surrounded by manufacturing industries, Newark Bay is a highly contaminated area with numerous sources (sewage treatment plants, National Pollutant Discharge Elimination System discharges, and nonpoint sources). This bay is downstream from a dioxin Superfund site that contains some of the highest dioxin concentrations in the United States and also is downstream from a mercury Superfund site. The dioxin concentration in the area sampled for this demonstration was approximately 450 pg/g. Average PCB concentrations ranged from 300 to 740 ppb. Fine-grained sediments make up 50% to 90% of the dredged material. Average total organic carbon (TOC) was about 4%.

3.2.2.1.2 Raritan Bay

Surrounded by industry and residential discharges, Raritan Bay has dioxin contamination in the area, but it is not to the degree of Newark Bay. No major Superfund sites are located in the vicinity. Dioxin concentration should be significantly less than in Newark Bay. PCB concentrations are around 250 ppb. The fine-grained sediment and TOC values were similar to percentages in Newark Bay.

3.2.2.2 Tittabawassee River

The first Tittabawassee River location was approximately ¼-mile upstream of the Bob Caldwell Boat Launch in Midland, Michigan. The sediments are dark gray, fine sand with some silt. The estimated TEQ concentration was 260 pg/g; however, concentrations as high as 2,100 pg/g TEQ have been found in this area. The second site was on the Tittabawassee River approximately 100 yards downstream from old Smith's Crossing Bridge in Midland, Michigan. The sediment was brown and sandy with organic material. The estimated TEQ concentration was 870 pg/g; but, again, concentrations as high as 2,100 pg/g TEQ are possible in the area. The third site was on Tittabawassee River at the Emerson Park Golfside Boat Launch. The sediment was gray black silty sand, with many leaves and high organic matter. The estimated TEQ concentration was < 5 pg/g. The fourth site was on the Tittabawassee River adjacent to Imerman Park in Saginaw County across from the fishing dock. The sediment was sand with some silt. The estimated TEQ concentration was between 100 and 2,000 pg/g TEQ. The fifth site was on the Tittabawassee River approximately 1 mile downstream of Center Road Boat Launch in Saginaw Township. The sediment consisted of sand and gravel with some shells and not much organic matter. The estimated TEQ concentration was between 100 and 1,000 pg/g TEQ. The sixth site also was on the Tittabawassee River across from the Center Road Boat Launch. The sediment was fine sand with high organic matter. The estimated TEQ concentration was 1,000 pg/g TEQ. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing.

3.2.2.3 Saginaw River

Saginaw River samples were collected at six locations. The first sampling location was in the Saginaw River just downstream of Green Point Island. Samples were collected near the middle of the river in about 21 feet of water. The sample was granular with some organic material. The estimated TEQ concentration was 100 ppt. Another Saginaw River sample was taken upstream of Genesee Bridge on the right side of the river. The sample was a brown fine sand from about 15 feet of water. The

estimated TEQ concentration was 100 ppt. The third location was in the Saginaw River downstream of the Saginaw wastewater treatment plant in about eight feet of water. The sample was gray silty clay with an unknown TEQ concentration. The fourth location was in the Saginaw River in about eight feet of water. The sample was a black sandy material. The estimated TEQ concentration for this location was unknown. The fifth location was downstream of a petroleum pipeline crossing upstream of the Detroit and Mackinaw railroad bridge crossing. This location was selected because of its proximity to a former PCB dredging location. The sediment sample consisted of dark black silt with some sand. The estimated TEQ concentration was unknown, but PCB concentrations are expected to be high. The sixth and final sampling location was near the mouth of the Saginaw River in about five feet of water. The sediment was a mix of fine black silt and layers of sand and shells. The estimated TEQ concentration for this location was also unknown.

3.2.2.4 Brunswick Wood Preserving Site

The Brunswick Wood Preserving Superfund site is located in Glynn County, Georgia, north of the city of Brunswick. The site was originally located in the city of Brunswick, but moved to its present location around 1958. The site is approximately 84 acres and is about two-thirds of a mile long. Burnett Creek, a tidally influenced stream, is located at the western corner of the site. At several points, most, if not all, of the drainage from the site flows into Burnett Creek. The site was first operated by American Creosote Company, which constructed the facility sometime between 1958 and 1960. The site was acquired by Escambia Treating Company in 1969 from Georgia Creosoting Company and the Brunswick Creosoting Company. In 1985, a corporate reorganization resulted in the purchase of the facility by the Brunswick Wood Preserving Company, which operated the site until it closed in early 1991. Each of the three major wood-treating operations was carried out at the facility: PCP, creosote, and chromium-copper-arsenic (CCA). The site was listed on the EPA's National Priorities List on April 1, 1997.

Sediment samples from the Brunswick Wood Preserving site in Brunswick, Georgia, were collected from six locations on the site, including areas thought to have lower (< 300 pg/g TEQ) and higher (> 10,000 pg/g TEQ) dioxin/furan (D/F) concentrations. Due to the processes that occurred on this site, the samples also contain varying levels of PAHs and PCP, but they were not expected to contain PCBs.

Chapter 4
Demonstration Approach

This chapter discusses the demonstration objectives, sample collection, sample homogenization, and demonstration design.

4.1 Demonstration Objectives

The primary goal of the SITE MMT Program is to develop reliable performance and cost data on innovative, commercial-ready technologies. A SITE demonstration must provide detailed and reliable performance and cost data so that technology users have adequate information to make sound decisions regarding comparability to conventional methods. The demonstration had both primary and secondary objectives. Primary objectives were critical to the technology evaluation and required the use of quantitative results to draw conclusions regarding a technology's performance. Secondary objectives pertained to information that is useful to know about the technology but did not require the use of quantitative results to draw conclusions regarding a technology's performance.

The primary objectives for the demonstration of the participating technologies were as follows:

P1. Determine the accuracy.
P2. Determine the precision.
P3. Determine the comparability of the technology to EPA standard methods.
P4. Determine the estimated method detection limit (EMDL).
P5. Determine the frequency of false positive and false negative results.
P6. Evaluate the impact of matrix effects on technology performance.
P7. Estimate costs associated with the operation of the technology.

The secondary objectives for the demonstration of the participating technologies were as follows:

S1. Assess the skills and training required to properly operate the technology.
S2. Document health and safety aspects associated with the technology.
S3. Evaluate the portability of the technology.
S4. Determine the sample throughput.

Application of these objectives to the demonstration was addressed based on input from the Dioxin SITE Demonstration Panel members,[2] general user expectations of field measurement technologies, the time available to complete the demonstration, technology capabilities that the developers participating in the demonstration intend to highlight, and the historical experimental components of former SITE Program demonstrations to maintain consistency.

Note that this demonstration does not assess all parameters that can affect performance of the technologies in comparison to the reference methods (i.e., not all Ah-receptor-inducing compounds have been characterized in the test samples, calibration of technologies results to HRMS results on site-by-site basis was not evaluated, etc.). However, the demonstration as outlined below was agreed upon by the Dioxin SITE Demonstration Panel members to provide a reasonable evaluation of the technologies.

4.2 Toxicity Equivalents

For risk assessment purposes, estimates of the toxicity of samples that contain a mixture of dioxin, furan, and PCB congeners are often expressed as TEQs. TEQs are calculated by multiplying the concentration of each congener with a toxicity equivalency factor (TEF), according to the equation:

$$TEQ = C_C * TEF$$

where C_C is the concentration of the congener. The TEF (see Table 4-1) provides an equivalency factor for each congener's toxicity relative to the toxicity of 2,3,7,8-TCDD. The TEFs used in this demonstration were determined by the World Health Organization (WHO) for mammalian species.(5) The total TEQ from dioxin and furans ($TEQ_{D/F}$) in a sample is calculated by adding up all of the TEQ values from the individual dioxin and furan congeners. The total TEQ contribution from PCBs (referred to as TEQ_{PCB}) is calculated by summing up the individual PCB TEQ values. The total TEQ in a sample is the sum of the $TEQ_{D/F}$ and TEQ_{PCB} values. TEQ concentrations for soils and sediments are typically reported in pg/g, which is equivalent to ppt.

Concentrations of dioxins, furans, and PCBs, represented as total TEQ concentration, provide a quantitative estimate of toxicity for all congeners expressed as if the mixture were a TEQ mass of 2,3,7,8-TCDD only. While the TEQ concept provides a way to estimate potential health or ecological effects, the limitations of this approach should be understood. The WHO report noted that the TEF indicates an order of magnitude estimate of the toxicity of a compound relative to 2,3,7,8-TCDD.(5) Therefore, the accuracy of the TEF factors could be affected by differences in species, in the functional responses elicited by the compounds, and in additive and nonadditive effects when the congeners are present in complex mixtures. The WHO report[5] concluded, however, that it is unlikely that a significant error would be observed due to these differences. The larger impact to the TEF concept is the presence of Ah-receptor binding compounds, such as PAHs (including naphthalenes, anthracenes, and fluorenes) and brominated and chloro/bromo-substituted analogues of PCDD/Fs that have not been assigned TEF values but which may contribute to the total TEQ. This potentially can result in an underestimation of TEQs in environmental samples using the TEF approach.(5)

Table 4-1. World Health Organization Toxicity Equivalency Factor Values

Compound[a]	WHO TEF	Compound	WHO TEF
PCDDs		**PCDFs**	
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDD	1	1,2,3,7,8-PeCDF	0.05
		2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDD	0.1	1,2,3,7,8,9-HxCDF	0.1
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1
		2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,4,6,7,8-HpCDF	0.01
		1,2,3,4,7,8,9-HpCDF	0.01
OCDD	0.0001	OCDF	0.0001
Dioxin-like PCBs			
Coplanar		**mono-*ortho***	
3,3',4,4'-TCB (PCB 77)	0.0001	2,3,3',4,4'-PeCB (PCB 105)	0.0001
3,4,4',5-TCB (PCB 81)	0.0001	2,3,4,4',5-PeCB (PCB 114)	0.0005
3,3',4,4',5-PeCB (PCB 126)	0.1	2,3',4,4',5-PeCB (PCB 118)	0.0001
3,3',4,4',5,5'-HxCB (PCB 169)	0.01	2,3,4,4',5-PeCB (PCB 123)	0.0001
		2,3,3',4,4',5-HxCB (PCB 156)	0.0005
		2,3,3',4,4',5-HxCB (PCB 157)	0.0005
		2,3',4,4',5,5'-HxCB (PCB 167)	0.00001
		2,3,3',4,4'5,5'-HpCB (PCB 189)	0.0001

[a] T = Tetra, Pe = Penta, Hx = Hexa, Hp = Hepta, O = Octa, CDD = chlorinated dibenzo-*p*-dioxin, CDF = chlorinated dibenzofuran, CB = chlorinated biphenyl

This demonstration was designed with these limitations of the TEQ concept in mind. The samples chosen contained a variety of combinations of dioxins, furans, and PCBs and at a wide range of concentration levels. Some samples were high in analytes with better understood TEFs, while others were high in analytes with TEFs that have more uncertainty. Some were high in other Ah-receptor binding compounds such as PAHs, while still others were free of these possible TEQ contributing compounds. The purpose was to evaluate each of the technologies under a variety of conditions and assess the comparability of the $TEQ_{D/F}$ and TEQ_{PCB} values determined by the reference laboratory.

4.3 Overview of Demonstration Samples

The goal of the demonstration was to perform a detailed evaluation of the overall performance of each technology for use in the field or mobile environment. The demonstration objectives were centered around providing performance data that support action levels for dioxin at contaminated sites. The Centers for Disease Control's Agency for Toxic Substances and Disease Registry (ATSDR) has established a decision framework for sites that are contaminated with dioxin and dioxin-like compounds.[6] If samples are determined to have dioxin TEQ levels between 50 and 1,000 pg/g, the site should be further evaluated; action is recommended for levels above 1,000 pg/g (i.e., 1 ppb) TEQ. A mix of PE samples, environmentally contaminated ("real-world") samples, and extracts were evaluated that bracket the ATSDR guidance levels. Table 4-2 lists the primary and secondary performance objectives for this demonstration and which sample types were used in each evaluation.

The PE samples were used primarily to determine the accuracy of the technology and consisted of purchased soil and sediment standard reference materials with certified concentrations of known contaminants and newly prepared spiked samples. The PE samples also were used to evaluate precision, comparability, EMDL, false positive/negative results, and matrix effects. Environmentally contaminated samples were collected from dioxin-contaminated sites around the country and were used to evaluate the precision, comparability, false positive/negative results, and matrix effects. Extracts, prepared in toluene, which was the solvent used by the reference laboratory, were used to evaluate precision, EMDL, and matrix effects. All samples were used to evaluate qualitative performance objectives such as technology cost, the required skill level of the operator, health and safety aspects, portability, and sample throughput. Table 4-3 shows the number of each sample type included in the experimental design. The following sections describe each sample type in greater detail.

4.3.1 PE Samples

PE standard reference materials are available through Cambridge Isotope Laboratories (CIL) (Andover, Massachusetts), LGC Promochem (United Kingdom), Wellington Laboratories (U.S. distributor TerraChem, Shawnee Mission, Kansas) the National Institute of Standards and Technology (NIST) (Gaithersburg, Maryland), and utilized to obtain PE samples for use in this demonstration, and Table 4-4 summarizes the PE samples that were included. PE samples consisted of three types of

Table 4-2. Distribution of Samples for the Evaluation of Performance Objectives

Performance Objective	Sample Type Used in Evaluation
P1: Accuracy	PE
P2: Precision	PE, environmental, extracts
P3: Comparability	PE, environmental, extracts
P4: EMDL	PE, extracts
P5: False positive/negative results	PE, environmental, extracts
P6: Matrix effects	PE, environmental, extracts
P7: Cost	PE, environmental, extracts
S1: Skill level of operator	PE, environmental, extracts
S2: Health and safety	PE, environmental, extracts
S3: Portability	PE, environmental, extracts
S4: Sample throughput	PE, environmental, extracts

Table 4-3. Number and Type of Samples Analyzed in the Demonstration

Sample Type	No. of Samples
PE	58
Environmental	128
Extracts	23
Total number of samples per technology	209

samples: (1) reference materials (RMs) or certified samples, which included soil and/or sediment samples with certified concentrations of dioxin, furan, and/or PCBs; (2) spiked samples, which included a certified dioxin, furan, PCB, and PAH-clean matrix spiked with known levels of dioxin and/or other contaminants; and (3) blank samples that were certified to have levels of dioxins, furans, WHO PCBs, and PAHs that were nondetectable or were considerably lower than the detection capabilities of developer technologies. The PE samples were selected based on availability and on the correlation of the PE composition as it related to the environmental samples that were chosen for the demonstration (e.g., the PE sample had a similar congener pattern to one or more of the environmental sites).

Table 4-4 indicates a correlation between the composition of the PE sample and the samples from the environmental sites, where applicable. The certified samples only required transfer from the original jar to the demonstration sample jar. The spiked samples were shipped to the characterization laboratory in bulk quantities so each had to be aliquoted in 50-g quantities. Additional details about each source of PE sample are provided further in this section.

4.3.1.1 Cambridge Isotopes Laboratories

Two RMs were obtained from CIL for use in this demonstration. RM 5183 is a soil sample that was collected from a location in Texas with the intended purpose of serving as an uncontaminated soil for use as a spiking material. The soil was sieved to achieve uniform particle size and homogenized to within 5% using a disodium fluorescein indicator. Samples were then sterilized three times for 2 hours at 121°C and 15 pounds per square inch (psi). Analytical results indicated that the soil had low levels of D/F and PCBs.

RM 5184 is a heavily contaminated soil sample with relatively high levels of D/F and PCBs. According to the Certificate of Analysis (CoA), approximately 75 kg of contaminated sediment were obtained from an EPA Superfund site in Massachusetts that was known to contain considerable contamination from PCBs and other chemical pollutants. The sediment was sieved to achieve uniform particle size and homogenized to within 5% using a disodium fluorescein indicator. Samples were then sterilized three times for 2 hours at 121°C and 15 psi.

RM 5183 and RM 5184 are newly available from CIL. For both RM 5183 and RM 5184, certified analytical values are provided for the D/F and the 12 WHO PCB congeners. The samples were included in an international interlaboratory study conducted by CIL and Cerilliant Corporation. More than 20 laboratories participated in analysis of the D/Fs; up to 20 laboratories participated in the analysis of the PCBs. Participating laboratories used a variety of sample preparation and analytical techniques.

4.3.1.2 LGC Promochem

Certified reference material (CRM) 529 was obtained from LGC Promochem. The following description is taken from the reference material report that accompanied CRM 529. The soil for CRM 529 was collected in Europe from a site where chloro-organic and other compounds had been in large-scale production for several decades, but where production had ceased more than five years before sampling. The site had been contaminated during long-term production of trichlorophenoxyacetic acid. An area of sandy soil was excavated to a depth of several meters. Several hundred kilograms of this mixed soil were air-dried at about 15°C for three months. After removal of stones and other foreign matter by sieving, the remaining material was

Table 4-4. Summary of Performance Evaluation Samples

Sample Type ID	Source	PE Type	Product No.	Certified Concentration			Correlation to Environ. Sample Type ID[a]	No. of Replicates Per Sample
				$TEQ_{D/F}$ (pg/g)	TEQ_{PCB} (pg/g)	PAH (mg/kg)		
PE #1	CIL	Certified	RM 5183	3.9	5.0	0.18	6	7[b]
PE #2	LGC Promochem	Certified	CRM 529	6,583	424[c]	NA[d]	5	4
PE #3	Wellington	Certified	WMS-01	62	10.5	NA	6	7[b]
PE #4	CIL	Certified	RM 5184	171	941	27	2, 8, 9	4
PE #5	NIST	Certified	SRM® 1944	251	41[c]	2.4[e]	3, 4	4
PE #6	ERA	Spiked	custom	11	NS[f]	< 0.33	10	4
PE #7	ERA	Spiked	custom	33	NS	< 0.33	10	4
PE #8	ERA	Spiked	custom	NS	NS	61[g]	5, 7	4
PE #9	ERA	Spiked	custom	NS	11	< 0.33	1	4
PE #10	ERA	Spiked	custom	NS	1,121	< 0.33	1	4
PE #11	ERA	Spiked	custom	11	3,760[c]	< 0.33	1	4
PE #12	ERA	Organic, Semivolatile, Blank Soil	056 (lot 56011)	0.046	0.01	< 0.33	not applicable	8
Total Number of PE samples								***58***

[a] Environmental Sample IDs are provided in Table 4-5.
[b] Seven replicates were analyzed for EMDL evaluation.
[c] Little or no certified PCB data were available; mean of reference laboratory measurements was used.
[d] NA = no data available.
[e] Approximate concentration of 2-methyl naphthalene, acenaphthene, and fluorene, which were the only PAHs that were included in the analysis.
[f] NS = not spiked.
[g] Each of the 18 target PAHs was spiked at levels that ranged from 1 to 10 mg/kg. (See Section 5.2.3 for the list of 18 PAHs.)

sterilized in air at 120°C for 2 hours, thoroughly mixed, and ground in an Alpine air jet mill to a particle size of <63 micrometers (μm). The material was homogenized once more in a Turbula mixer and packaged in 50-g quantities. The final mean moisture content at the time of bottling was found to be 1.5%. According to the CoA, certified values are provided for five dioxin congeners, seven furan congeners, three chlorobenzene compounds, and three chlorophenol compounds. No PCBs were reported with certified values on the CoA, so the mean concentration determined by the reference laboratory was used as the certified value.

4.3.1.3 Wellington

PE sample WMS-01 was obtained from TerraChem, the U.S. distributor for Wellington, an Ontario-based company. As described in the CoA, WMS-01 is a homogeneous lake sediment that was naturally contaminated (and not fortified). The crude, untreated sediment used to prepare WMS-01 was collected from Lake Ontario. The sediment obtained was subsequently air-dried; crushed to break up agglomerates; air-dried again, and then sieved, milled, and re-sieved (100% < 75 μm). The sediment was then subsampled into 25-g aliquots. The demonstration samples for only the Wellington PE samples were 25 g rather than 50 g based on the packaging size that was available from Wellington. Certified values for the 17 D/F congeners and the 12 WHO PCB congeners are provided on the CoA.

4.3.1.4 National Institute for Standards and Technology

Standard Reference Material® (SRM) 1944 was purchased through NIST. As described in the CoA, SRM 1944 is a mixture of marine sediment collected from six sites in the vicinity of New York Bay and Newark Bay in October 1994. Site selection was based on contaminant levels measured in previous samples from these sites and was intended to provide relatively high concentrations for a variety of chemical classes of contaminants. The sediment was collected using an

epoxy-coated modified Van Veen-type grab sampler designed to sample the sediment to a depth of 10 centimeters. A total of approximately 2,100 kg of wet sediment was collected from the six sites. The sediment was freeze-dried, sieved (nominally 61 to 250 μm), homogenized in a cone blender, radiation sterilized, then packaged in 50-g quantities. Certified values are provided on the CoA for the 17 D/F congeners, 30 PCB congeners, 24 PAHs, four chlorinated pesticides, 36 metals, and TOC. Since only three WHO PCBs were reported out of the 30 PCB congeners, the mean concentration of the reference laboratory measurements was used as the certified value so that the TEQ_{PCB} concentration would not be underestimated when compared to the developer technologies.

4.3.1.5 Environmental Resource Associates

ERA synthesized PE samples for this demonstration. ERA spiked blank, uncontaminated soil to pre-determined levels of D/Fs, PCBs, and/or PAHs. Spiked PE samples were prepared to include additional concentration ranges and compositions that were not covered with the commercially available certified materials. The organic semivolatile soil blank (ERA Product #056, Lot 56011) is a topsoil that was obtained from a nursery and processed according to ERA specifications by a geochemical laboratory. The particle size distribution of the soil was -20/+60 mesh. The soil was processed and blended with a sandy loam soil to create a blank soil with the following make-up: 4.1% clay, 4.5% silt, 91.2% sandy and 0.2% organic material. Initially, ERA was required to certify that the blank soil matrix to be used as the blank and for the preparation of the spiked PE samples was "clean" relative to the list of required target analytes. This was accomplished through a combination of ERA-conducted analyses (PAHs, pesticides, semivolatile organic compounds, and Aroclors, which are trade mixtures of PCB congeners) and subcontracted analytical verification (D/F and PCBs). The subcontracted analyses were performed by Alta Analytical Perspectives, LLC, in Wilmington, North Carolina. The Alta Analytical Certificate of Results and the ERA Certification sheets for the organic semivolatile soil blank indicated that trace levels of the octa-dioxins and several WHO PCB congeners were detected, but the total TEQ (combined D/F and PCBs) was less than 0.06 pg/g. The level of PAHs, pesticides, Aroclors, and semivolatile organic compounds in the soil was determined to be < 0.33 pg/g. The TEQ level was considerably below the detection capabilities of the participating technologies, so the organic semivolatile soil blank was considered adequately clean for use in this demonstration.

The manufacturing techniques that ERA used to prepare the PE samples for this demonstration were consistent with those used for typical semivolatile soil products by ERA. These techniques have been validated through hundreds of round robin performance test studies over ERA's more than 25 years in business. The D/F stock solutions used in the manufacture of these PE samples were purchases from CIL. The PCB and PAH stock solutions were purchased from ChemService. For each PE sample, a spiking concentrate was prepared by combining appropriate weight/volume aliquots of stock materials required for that PE sample. Typically, additional solvent was added to this concentrate to yield sufficient volume of solution, appropriate for the mass of soil to be spiked. Based on a soil mass of 1,600 g, the volume of spike concentrate was approximately 10 to 30 milliliter (mL). For each PE sample, the blank soil matrix was weighed into a 2-liter (L) wide mouth glass jar, the spike concentrate was distributed onto the soil, and the soil was allowed to air-dry for 30 to 60 minutes. The PE samples were then capped and mixed in a rotary tumbler for 30 minutes. Each PE sample was certified as the concentration of target analytes present in the blank matrix, plus the amount added during manufacture, based on volumetric and gravimetric measurements. CoAs were provided by ERA for all six ERA-provided PE samples. The certified values provided by ERA were different from the commercially available certified samples since the data were not based on analytically derived results. Further confirmation of the concentrations was conducted by the reference laboratory.

4.3.2 Environmental Samples

Handling of the environmental samples is described in this section. Note that once the environmental samples were collected, they were dried and homogenized as best as possible to eliminate variability introduced by sample homogeneity. As such, the effect of moisture on the sample analysis was not investigated.

4.3.2.1 Environmental Sample Collection

Samples were collected by the EPA, an EPA contractor, or MDEQ and shipped to the characterization laboratory. When determining whether a soil or sediment site had appropriate dioxin contamination, a guideline concentration range of < 50 pg/g to 5,000 pg/g was used.

Once necessary approvals and sampling locations had been secured, sample containers were shipped to site personnel. Each site providing samples received 1-gallon containers (Environmental Sampling Supply, Oakland, California, Part number 3785-1051, wide-mouth, 128-ounce high-density polyethylene round packer) for collecting five or six samples.

Instructions for sample collection, as well as how the containers were to be labeled and returned, were included in a cover letter with the sample containers that were shipped to each site. Personnel collecting the samples were instructed to label two containers containing the same sample as "1 of 2" and "2 of 2" and to attach a description or label to each container with a description of the sample, including where the sample was collected and the estimated concentrations of dioxin and any other anticipated contamination (e.g., PCBs, PAHs, PCP). Final instructions to sample providers indicated that collected samples were to be shipped back to the characterization laboratory using the provided coolers. Federal Express labels that included an account number and the shipping address were enclosed in each shipment.

Sample providers also were asked to provide any information about the possible source of contamination or any historical data and other information, such as descriptions of the sites, for inclusion in the D/QAPP.

4.3.2.2 Homogenization of Environmental Samples

If the material had very high moisture content, the jar contents were allowed to settle, and the water was poured off. Extremely wet material was poured through fine mesh nylon material to remove water. After water removal, the material was transferred to a Pyrex™ pan and mixed. After thorough mixing, an aliquot was stored in a pre-cleaned jar as a sample of "unhomogenized" material and was frozen.[1] The remaining bulk sample was mixed and folded bottom to top three times. This material was split equally among multiple pans. In each pan, the material was spread out to cover the entire bottom of the pan to an equal depth of approximately 0.5 inches. The pans were placed in an oven at 35ºC and held there until the samples were visibly dry. This process took from 24 to 72 hours, depending on the sample moisture. The trays were removed from the oven and allowed to rise to room temperature by sitting in a fume hood for approximately 2 hours. Approximately 500 g of material were put in a blender and blended for 2 minutes. The blender sides were scraped with a spatula and the sample blended for a second 2-minute period. The sample was sieved [USA Standard testing, No. 10, 2.00-millimeter (mm) opening] and the fine material placed in a tray. Rocks and particles that were retained on the sieve were placed in a pan. This process was repeated until all of the sediment or soil were blended and sieved. The blended and sieved sediment or soil in the tray was mixed well, and four aliquots of 100- to 300-g each were put into clean jars (short, wide-mouth 4-ounce, Environmental Sampling Supply, Oakland, California, Part number 0125-0055) to be used for the characterization analyses. The remaining sediment or soil was placed in a clean jar, and the particles that were retained on the sieve were disposed of. The jars of homogenized sediment and soil were stored frozen (approximately -20°C), unless the samples were being used over a period of several days, at which time they were temporarily stored at room temperature.

4.3.2.3 Selection of Environmental Samples

Once homogenized, the environmental samples were characterized for dioxin/furans (EPA Method 1613B[3]), PCBs, low-resolution mass spectrometry (LRMS), modified EPA Method 1668A[4], and 18 target PAHs [National Oceanic and Atmospheric Administration (NOAA) method[7]] to establish the basic composition of the samples. (Characterization analyses are described in Chapter 5.) Because the soil and sediment samples were dried and homogenized, they were indistinguishable. As such, the soil and sediment samples were jointly referred

[1] Ideally, the samples would have been stored at 4° ± 2°C; but, due to the large volume of buckets and jars that needed to be stored, the most adequate available storage at the characterization laboratory was a walk-in freezer that was at approximately minus 20°C.

to as "environmental" samples, with no distinction made between soil or sediment, other than during the matrix effects evaluations, as described in Section 4.7.6. Environmental samples were selected for inclusion in the demonstration based on the preliminary characterization data. The number and type of samples from each sampling location included in the demonstration are presented in Table 4-5.

Four aliquots of the homogenized material and one aliquot of unhomogenized material were analyzed. Two criteria had to be met for the environmental sample to be considered for inclusion in the demonstration. The first criterion was that the relative standard deviation (RSD) of the total D/F TEQ values from the four aliquots had to be less than 20% for samples with total TEQ values > 50 pg/g; RSD values up to 30% were considered acceptable if the concentration was < 50 pg/g TEQ. The second criterion was that no single RSD for an individual congener could be greater than 30%. If both of these criteria were met, the sample met the homogenization criteria and was considered for inclusion in the demonstration. If either of these criteria was not met, options for the sample included (a) discarding it and not considering it for use in the demonstration, (b) reanalyzing it to determine if the data outside the homogenization criteria were due to analytical issues, or (c) rehomogenizing and reanalyzing it. Of these options, (a) and (b) were utilized, but (c) was not because an adequate number of environmental samples were selected using criteria (a) and (b). The average D/F concentration and RSDs for the homogenization analyses of environmental samples are shown in Table 4-5. The composition of two particular Saginaw River samples was of interest for inclusion in the demonstration because of their concentration and unique congener pattern, but the homogenization criteria were slightly exceeded (i.e., 28% and 34% RSD for Saginaw River Sample #2 and Saginaw River Sample #3, respectively). Since multiple replicates of every sample were analyzed, those samples were included in the study because of their unique nature, but are flagged as slightly exceeding the homogenization criteria. A correlation of environmental samples to PE samples, similar to that presented in Table 4-4, is presented in Table 4-5.

4.3.3 Extracts

A summary of the extract samples is provided in Table 4-6. The purpose of the extract samples was to evaluate detection and measurement performance independent of the sample extraction method. As shown in Table 4-6, two environmental samples (both sediments) were extracted using Soxhlet extraction with toluene. These extractions were performed by AXYS Analytical Services consistent with the procedures to extract the demonstration samples for reference analyses.[2] The environmental sample extracts represented a 10-g sediment sample extraction and were reported in pg/mL, which was calculated by the following equation:

$$\text{pg/mL} = \frac{(\text{pg/g samples}) \times (10\,\text{g aliquot})}{(300\,\text{mL extraction volume})} \times (30\,\text{DF})$$

where DF = dilution factor.

Total extract volume per 10-g aliquot was 300 mL, but the sample extracts were concentrated and provided to the developers as 10-mL extracts, so a 30x dilution factor is included. The extracts were not processed through any cleanup steps, but they were derived from sediment samples that also were included in the suite of environmental samples. All environmental sample extractions were prepared in the same solvent (toluene). The extract samples also included three toluene-spiked solutions that were not extractions of actual environmental samples. Because adequate homogenization at trace quantities was difficult to achieve, one set of extract samples was spiked at low levels (approximately 0.5 pg/mL of 2,3,7,8-TCDD) and used as part of the EMDL evaluation.

4.4 Sample Handling

In preparation for the demonstration, the bulk homogenized samples were split into jars for distribution. Each 4-ounce, amber, wide-mouth glass sample jar (Environmental Sampling Supply, Oakland, California, Part number 0125-0055) contained approximately 50 g of sample. Seven sets of samples were prepared for five developers, the reference laboratory, and one archived set. A minimum of four replicate splits of each sample was prepared for each participant, for a total of at least 28 aliquots prepared for each sample. The purchased PE samples (i.e., standard reference materials and spiked materials) were transferred from their original packaging to the jars to be used in the demonstration for the environmental samples making

Table 4-5. Characterization and Homogenization Analysis Results for Environmental Samples

Sample Type ID	Environmental Site Location	Soil or Sediment	Sample No.	Average Total $TEQ_{D/F}$ Concentration (pg/g)	RSD (%)	No. of Replicates Per Sample	Correlation with PE Sample Type ID[a]
Env Site #1	Warren County, North Carolina	soil	1	274	11	4	9, 10, 11
			2	5,065	7	4	
			3	11,789	3	4	
Env Site #2	Tittabawassee River, Michigan	soil	1	42	23[b]	4	4
			2	435	5	4	
			3	808	10	4	
Env Site #3	Newark Bay, New Jersey	sediment	1	16	26[b]	4	5
			2	62	14	4	
			3	45	26[b]	4	
			4	32	6	4	
Env Site #4	Raritan Bay, New Jersey	sediment	1	12	2	4	5
			2	14	3	4	
			3	13	7	4	
Env Site #5	Winona Post, Missouri	soil	1	3,831	1	4	2, 8
			2	11,071	2	4	
			3	11,739	1	4	
Env Site #6	Tittabawassee River, Michigan	sediment	1	1	23[b]	4	1, 3
			2	55	7	4	
			3	16	26[b]	4	
Env Site #7	Brunswick, Georgia	sediment	1	69	8	4	8
			2	65	1	4	
			3	14,500	2	4	
Env Site #8	Saginaw River, Michigan	sediment	1	921	9	4	4
			2	1,083	28[c]	4	
			3	204	34[c]	4	
Env Site #9	Midland, Michigan	soil	1	239	5	4	4
			2	184	5	4	
			3	149	7	4	
			4	25	10	4	
Env Site #10	Solutia, West Virginia	soil	1	48	10	4	6, 7
			2	1,833	19	4	
			3	3,257	11	4	
Average RSD for all environmental samples used in demonstration						***11%***	
Total number of environmental samples						***128***	

[a] PE Sample IDs are provided in Table 4-4.

[b] RSD values up to 30% were allowed for samples where the characterization analyses determined concentration to be <50 pg/g total $TEQ_{D/F}$.

[c] RSD value slightly exceeded the homogeneity criteria, but samples were included in the demonstration because they were samples of interest.

the environmental and PE samples visually indistinguishable.

The samples were randomized in two ways. First, the order in which the filled jars were distributed was randomized. All jars had two labels. The label on the top of the jar was the analysis order and contained sample numbers 1 through 209. A second label placed on the side of the jar contained a coded identifier including a series of 10 numbers coded to include the

Table 4-6. Distribution of Extract Samples

Sample Type ID	Sample ID	Sample Description	No. of Replicates per Sample
Extract #1	Environmental #6, Sample #2	Soxhlet extraction in toluene; no cleanup	4
Extract #2	Environmental #7, Sample #1	Soxhlet extraction in toluene; no cleanup	4
Extract #3	Spike #1[a]	0.5 pg/mL 2,3,7,8-TCDD	7[b]
Extract #4	Spike #2[a]	100 pg/mL 2,3,7,8-TCDD 1,000 pg/mL each WHO PCB (TEQ ~ 11)	4
Extract #5	Spike #3[a]	10,000 pg/mL each WHO PCB[c] (TEQ ~ 1,000)	4
Total number of extracts			***23***

[a] Prepared in toluene.
[b] Seven replicates were analyzed for EMDL evaluation.
[c] This extract was spiked with only PCBs, but a low-level (approximately 0.3 pg/mL) 2,3,7,8-TCDD contamination was confirmed by the reference laboratory.

site, replicate, developer, and matrix. All samples believed to have at least one D/F or PCB congener greater than 10,000 pg/g were marked with an asterisk for safety purposes. This was consistent for both the developer and reference laboratory samples. The developer was given the option of knowing which environmental site the samples came from and whether the sample was a soil or sediment. XDS elected not to have any of this information. As described in the D/QAPP, AXYS was informed of which environmental site that the samples came from so it could use congener profiles and dilution schemes determined during the pre-demonstration phase as a guide, along with the concentration range data that was provided in the D/QAPP. This information was supplied to the reference laboratory with the samples, along with which samples contained high (i.e., a sample with at least one congener with concentration > 120,000 pg/g) or ultrahigh (i.e., a sample with at least one congener with concentration > 1,200,000 pg/g) PCB levels. Using this information, AXYS regrouped the samples in batches so that, to the extent possible, samples from the same site would be analyzed within the same analytical batch. Because an analytical laboratory might know at least what site samples came from, and because it is reasonable from an analytical standpoint to group samples that might require similar dilution schemes and which have similar congener patterns in an analytical batch, this approach was an acceptable deviation from the original intention of having the samples run by the reference laboratory completely blind and in the prescribed analytical order. XDS analyzed the samples in the order received. The extracts were the first 23 samples in the XDS analysis order.

The environmental samples were stored at room temperature until homogenized. After homogenization and prior to distribution during the demonstration, the samples were stored in a walk-in freezer (approximately -20 °C) at the characterization laboratory. At the demonstration site, the samples were stored at ambient temperature. After the demonstration analyses were completed, the samples were stored at the character-ization laboratory in the walk-in freezer until the conclusion of the project.

4.5 Pre-Demonstration Study

Prior to the demonstration, pre-demonstration samples were sent to XDS for evaluation in its laboratory. The pre-demonstration study comprised 15 samples, including PE samples, environmental samples, and extracts. The samples selected for the pre-demonstration study covered a wide range of concentrations and

included a representative of each environmental site analyzed during the demonstration.

The pre-demonstration study was conducted in two phases. In Phase 1, XDS was sent six soil/sediment samples with the corresponding D/F, PCB, and PAH characterization data to perform a self-evaluation of the CALUX® by XDS assay. In Phase 2, seven additional soil/sediment samples and two extracts were sent to XDS for blind evaluation. AXYS analyzed all 15 pre-demonstration samples blindly. The XDS pre-demonstration results were paired with the AXYS results and returned to XDS so they could use the HRMS pre-demonstration sample data to refine the performance of the CALUX® by XDS assay prior to participating in the field demonstration. Results for the pre-demonstration study can be found in the DER, which can be obtained by contacting the EPA program manager for this demonstration. The results confirmed that XDS was a viable candidate to continue in the demonstration process.

4.6 Execution of Field Demonstration

XDS arrived on-site on Sunday, April 25, and spent several hours setting up its mobile laboratory. The demonstration officially commenced on Monday, April 26 after 1.5 hours of safety and logistical training. During this meeting, the health and safety plan was reviewed to ensure that all participants understood the safety requirements for the demonstration. Logistics, such as how samples would be distributed and results reported, were also reviewed during this meeting. After the safety and site-specific training meeting and prior to samples being received by the developers, each trailer and mobile laboratory was surface-wipe-sampled on the floor to the entrance of the developer work area to establish the background level of D/F and PCB contamination. The wipe sampling procedure was followed as described in the D/QAPP.[2] Following demobilization by the developers, all of the trailers and mobile laboratories were cleaned and surface-wipe-sampled. Analysis of the pre- and post-deployment wipe samples indicated that all trailers and mobile laboratories met the acceptable clearance criteria that were outlined in the D/QAPP. Only one fume hood had to be re-cleaned and re-sampled before receiving final clearance.

Ideally, all 209 demonstration samples would have been analyzed on-site, but sample throughput of some of the technologies participating in the demonstration would require three weeks or more in the field to analyze 209 samples. Consequently, it was decided, as reported in the D/QAPP, that the number of samples to be analyzed in the field by each developer would be determined at the discretion of the developer.

XDS received its first batch of samples by midmorning on April 26. XDS completed analysis of 43 samples (23 extracts and 20 soil/sediment samples) in 5 working days (on April 30). It should be noted that the morning of April 28 was dedicated to a Visitor's Day, so minimal work on sample analyses was performed. XDS also encountered some equipment failures that were not the fault of the developer that impeded progress. These are described in detail in Section 7.2. The remaining 166 samples were completed by XDS in its laboratories. These samples were shipped to XDS on May 3 and received at XDS on May 4. The remaining 166 samples analyzed in the XDS laboratories were reported on June 16. XDS reports that typical (nonexpedited) turn around times for sample analyses in their laboratory is 21 to 30 days. Once the complete data set was submitted, XDS was offered the opportunity to reanalyze any samples before reporting final results, but it declined this offer and elected to not re-run any of the samples.

4.7 Assessment of Primary and Secondary Objectives

The purpose of this section is to describe how the primary and secondary objectives are assessed, as presented in Chapters 6 and 7.

XDS reported its results in $TEQ_{D/F}$ and TEQ_{PCB} (both in pg/g). The XDS results were compared to the certified values and reference laboratory results for $TEQ_{D/F}$, TEQ_{PCB}, and total TEQ. For the developer data, total TEQ values were calculated by summing $TEQ_{D/F}$ and TEQ_{PCB} data. If one of the values was reported as a nondetect (i.e., "< reporting limits") or was not reported (i.e., "NA"), a value of zero was used. In the case where one of the values was reported as, "> reporting limit", the reporting limit value was used. If both values were, "< reporting limits", "> reporting limits", and/or "NA", a total TEQ value could not be calculated. For the reference laboratory data, total TEQ values were calculated for all samples except for two which were excluded due to sample preparation issues (see Section 6.4).

4.7.1 Primary Objective P1: Accuracy

The determination of accuracy was based on agreement with certified or spiked levels of PE samples. PE samples containing concentrations from across the analytical range of interest were analyzed. Percent recovery values relative to the certified or spiked concentrations were calculated. To evaluate accuracy, the average of replicate results from the field technology measurement was compared to the certified or spiked value of the PE samples to calculate percent recovery. The equation used was:

$$R = \overline{C} / C_R \times 100\%$$

where $\overline{C}$ is the mean concentration value calculated from the technology replicate measurements (in pg/g TEQ) and C_R is the certified value (in pg/g TEQ). Nondetects and values reported as "> (value)" were not included in the accuracy assessment. Mean concentration values were determined when at least three of four replicates were reported as actual values [i.e., were not reported as, "< (value)" or "> (value)"]. The mean, median, minimum, and maximum R values are reported as an assessment of overall accuracy. An ideal R value would be 100%.

4.7.2 Primary Objective P2: Precision

To evaluate precision, all samples (including PE, environmental, and extract samples) were analyzed in at least quadruplicate. Seven replicates of three different samples were analyzed to evaluate EMDLs.

Precision was evaluated at both low and high concentration levels and across different matrices. The statistic used to evaluate precision was RSD. The equation used to calculate standard deviation (*SD*) between replicate measurements was:

$$SD = \left[\frac{1}{n-1} \sum_{k-1}^{n} \left(\overline{C}_k - \overline{C} \right)^2 \right]^{1/2}$$

where *SD* is the standard deviation and $\overline{C}$ is the average measurement. Both are reported in pg/g TEQ.

The equation used to calculate RSD, reported in percent, between replicate measurements was:

$$\text{RSD} = \left| \frac{\text{SD}}{\overline{\text{C}}} \right| \times 100\%$$

RSD was calculated if detectable concentrations were reported for at least three replicates. The mean, median, minimum, and maximum RSD values are reported as an assessment of overall precision.

Low RSD values (< 20%) indicated high precision. For a given set of replicate samples, the RSD of results was compared with that of the laboratory reference method's results to determine whether the reference method is more precise than the technology or vice versa for a particular sample set. The mean RSD for all samples was calculated to determine an overall precision estimate.

4.7.3 Primary Objective P3: Comparability

Data comparability was maximized by using the homogenization procedures and applying criteria for acceptable results prior to a sample being included in the demonstration. (See Section 4.3.2.3 for additional information.)

Technology results reported by XDS were compared to the corresponding reference laboratory results by calculating a relative percent difference (RPD). The equation for RPD, reported in percent, is as follows:

$$\text{RPD} = \frac{\left(\text{M}_\text{R} - \text{M}_\text{D} \right)}{\text{average}\left(\text{M}_\text{R}, \text{M}_\text{D} \right)} \times 100\%$$

where M_R is the reference laboratory measurement (in pg/g TEQ) and M_D is the developer measurement (in pg/g TEQ). Nondetects were not included in this evaluation. Because the CALUX® by XDS reported both $TEQ_{D/F}$ and TEQ_{PCB} values, the XDS results were compared to the reference laboratory $TEQ_{D/F}$ and TEQ_{PCB} as well as the total TEQ values. For PE samples, $TEQ_{D/F}$ and TEQ_{PCB} RPD calculations were only performed for the analyte classes that the PE sample contained. For example, PE sample #6 was only spiked with 2,3,7,8-TCDD. Consequently, RPD calculations were only performed for $TEQ_{D/F}$ and not TEQ_{PCB} or total TEQ.

The absolute value of the difference between the reference and developer measurements in the equation above was not taken so that the RPD would indicate whether the technology measurements were greater than the reference laboratory measurements (negative RPD values) or less than the reference laboratory measurements (positive RPD). Because negative values for RPD could be obtained with this approach, the median RPD of all individual RPDs was calculated rather than the average

RPD in calculation of comparability between the XDS results and reference laboratory measurements. The median, minimum, and maximum RPD values were reported as an assessment of overall comparability. RPD values between positive and negative 25% indicated good agreement between the two measurements.

As another measure of comparability, the developer and reference data were grouped into four TEQ concentration ranges. The ranges were ≤ 50 pg/g, 50 to 500 pg/g, 500 to 5,000 pg/g, and ≥ 5,000 pg/g. The intervals were determined by the Demonstration Panel and were based on current guidance for cleanup levels. The percentage of developer results that agreed with those ranges of values was reported.

The accuracy of reporting blank samples was assessed. The blanks included eight replicate samples that contained levels of D/Fs and PCBs that were below the reporting limits of the developer technology but contained levels that could be detected by the reference methods (see Table 4-4). If the reference laboratory result was in the nondetect interval reported by the developer technology reporting limit, this result was considered accurately reported by the developer. The accuracy of the blank samples was reported in terms of % agreement. Ideal % agreement values would be 100%.

4.7.4 Primary Objective P4: Estimated Method Detection Limit

The method detection limit (MDL) calculation procedure described in the demonstration plan was 40 CFR Part 136, Appendix B, Revision 1.11. This procedure is based on an assumption that the replicates are homogeneous enough to allow proper measurement of the analytical precision and that the concentration is in the appropriate range for evaluation of the technology's sensitivity. For this evaluation, XDS analyzed seven aliquots each of a low-level PE soil, PE sediment, and a toluene-spiked extract. MDL-designated samples are indicated in Tables 4-4 and 4-6. The developer reported nondetect values for some of the replicates, so provisions had to be made for the treatment of nondetects. As such, the results from these samples were used to calculate an estimated MDL (EMDL) for the technology.

A Student's t-value and the standard deviation of seven replicates were used to calculate the EMDL in pg/g TEQ is shown in the following equation:

$$EMDL = t_{(n-1,1-\infty=0.99)}(SD)$$

where $t_{(n-1,1-\infty=0.99)}$ = Student's t-value appropriate for a 99 percent confidence level and a standard deviation estimate with n-1 degrees of freedom. Nondetect values were assigned the reported value (i.e., "< 1" was assigned as value of 1), half of the reported value (i.e., "< 1" was assigned 0.5), or zero. The various treatments of nondetect values were performed to see the impact that reduced statistical power (i.e., lower degrees of freedom) had on the EMDL calculation. The lower the EMDL value, the more sensitive the technology is at detecting contamination.

4.7.5 Primary Objective P5: False Positive/False Negative Results

The tendency for the CALUX® by XDS to return false positive results (e.g., results reported above a specified level for the field technology but below a specified level by the reference laboratory) was evaluated. The frequency of false positive results was reported as a fraction of results available for false positive analysis. Similarly, the frequency of false negatives results was examined. For this purpose, the results were evaluated for samples reported as having concentrations above and below 1 pg/g TEQ and above and below 50 pg/g TEQ. As such, the samples that were reported as ≤ 1 (or 50) pg/g TEQ by the reference laboratory but > 1 (or 50) pg/g TEQ by XDS were considered false positive. Conversely, those samples that were reported as ≤ 1 (or 50) pg/g TEQ by XDS, but reported as > 1 (or 50) pg/g TEQ by the reference laboratory, were considered false negatives. In the case of semiquantitative results (reported as < or >), if the laboratory result was within the interval reported by the developer, it was not considered a false positive or false negative result. Ideal false positive and negative percentages would be equal to zero.

4.7.6 Primary Objective P6: Matrix Effects

The likelihood of matrix-dependent effects on performance was investigated by grouping the data by matrix type (i.e., soil, sediment, extract), sample type (i.e., PE, environmental, and extract), varying levels of PAHs, environmental site, and known interferences.

Precision (RSD) data were summarized by soil, sediment, and extract (matrix type); by environmental, PE, and extract (sample type); and by PAH concentration. Analysis of variance (ANOVA) tests were performed to determine if there was a dependence on matrix type or sample type. Only the environmental samples were included in the matrix effect assessment based on PAH concentration, because only the environmental samples were analyzed for PAHs during the characterization analysis (described in Section 5.2.3). Some PAH data were available for the PE samples, but data were not available for all of the same analytes that were determined during the characterization analysis. The environmental samples were segregated into four ranges of total PAH concentrations: < 1,000 nanogram/g (ng/g), 1,000 to 10,000 ng/g, 10,000 to 100,000 ng/g, and > 100,000 ng/g. The precision (RSD) data were summarized for samples within these PAH concentration ranges. ANOVA tests were used to determine if the summary values for RSD were statistically different, indicating performance dependent upon PAH concentration. For the environmental site evaluation, the comparability (RPD) values from each of the 10 environmental sites were compared to see if the developer results were more or less comparable to the reference laboratory for a particular site. For known interferences, the developer's reported results for PE samples were summarized for samples where the PE samples did not contain the target analyte (e.g., did the developer report D/F detections for a sample only spiked with PCBs).

This objective also evaluated whether performance was affected by measurement location (i.e., in-field versus laboratory conducted measurements), although this is not a traditional matrix effect. To evaluate the effect of measurement location, ANOVA tests were performed for sample results within a replicate set that were generated both in the laboratory and in the field. For these analyses, p-values < 0.05 indicated statistically different results between the laboratory and field measurements and therefore a significant effect of the measurement location on results. The percentage of replicate sets having p-values < 0.05 was reported.

4.7.7 Primary Objective P7: Technology Costs

The full cost of each technology was documented and compared to typical and actual costs for D/F and PCB reference analytical methods. Cost inputs included equipment, consumable materials, mobilization and demobilization, and labor. The evaluation of this objective is described in Chapter 8, Economic Analysis.

4.7.8 Secondary Objective S1: Skill Level of Operator

Based on observations during the field demonstration, the type of background and training required to properly operate the CALUX® by XDS was assessed and documented. The skill required of an operator was also evaluated. The evaluation of this secondary objective also included user-friendliness of the technology.

4.7.9 Secondary Objective S2: Health and Safety Aspects

Health and safety issues, as well as the amount and type of hazardous and nonhazardous waste generated, were evaluated based on observer notes during the field demonstration. This also included an assessment of the personal protective equipment required to operate the technology.

4.7.10 Secondary Objective S3: Portability

Observers documented whether the CALUX® by XDS could be readily transported to the field and how easy it was to operate in the field. This included an assessment of what infrastructure requirements were provided to XDS (e.g., a mobile laboratory) and an assessment of whether the infrastructure was adequate (or more than adequate) for the technology's operation. Limitations of operating the technology in the field are also discussed.

4.7.11 Secondary Objective S4: Sample Throughput

Sample throughput was measured based on the observer notes, which focused on the time-limiting steps of the procedures, as well as the documentation of sample custody. The number of hours XDS worked in the field was documented using attendance log sheets where XDS recorded the time they arrived and departed from the demonstration site. Time was removed for training and Visitor's Day activities. The number of operators involved in the sample analyses also was noted. Throughput of the developer technology was compared to that of the reference laboratory.

Chapter 5
Confirmatory Process

This chapter describes the characterization analyses and the process for selecting the reference methods and the reference laboratory.

5.1 Traditional Methods for Measurement of Dioxin and Dioxin-Like Compounds in Soil and Sediment

Traditional methods for analysis of dioxin and dioxin-like compounds involve extensive sample preparation and analysis using expensive instrumentation resulting in very accurate and high-quality, but costly, information. The ability to use traditional methods for high-volume sampling programs or screening of a contaminated site often is limited by budgetary constraints. The cost of these analyses can range approximately from $500 to $1,100 per sample per method, depending on the method selected, the level of quality assurance/quality control (QA/QC) incorporated into the analyses, and the reporting requirements.

5.1.1 High-Resolution Mass Spectrometry

EPA Method 1613B[3] and SW846 Method 8290[8] are both appropriate for low and trace-level analysis of dioxins and furans in a variety of matrices. They involve matrix-specific extraction, analyte-specific cleanup, and high-resolution capillary GC (HRGC)/HRMS analysis. The main differences between the two methods are that EPA Method 1613B has an expanded calibration range and requires use of additional $^{13}C_{12}$-labeled internal standards resulting in more accurate identifications and quantitations. The calibration ranges for the HRMS methods based on a typical 10-g sample and 20-microliter (µL) final sample volume are presented in Table 5-1.

Table 5-1. Calibration Range of HRMS Dioxin/Furan Method

Compound	EPA Method 1613B	SW846 Method 8290
Tetra Compounds	1–400 pg/g	2–400 pg/g
Penta-Hepta Compounds	5–2,000 pg/g	5–1,000 pg/g
Octa Compounds	10–4,000 pg/g	10–2,000 pg/g

5.1.2 Low-Resolution Mass Spectrometry

SW846 Method 8280 is appropriate for determining dioxins and furans in samples with relatively high concentrations, such as still bottoms, fuel oils, sludges, fly ash, and contaminated soils and waters. This method involves matrix specific extraction, analyte-specific cleanup, and HRGC/LRMS analysis. The calibration ranges in Table 5-2 are based on a typical 10-g sample size and 100-µL final volume.

Table 5-2. Calibration Range of LRMS Dioxin/Furan Method

Compound	SW846 Method 8280
Tetra-Penta Compounds	1,000–20,000 pg/g
Hexa-Hepta Compounds	2,500–50,000 pg/g
Octa Compounds	5,000–100,000 pg/g

5.1.3 PCB Methods

There are more options for analysis of dioxin-like compounds such as PCBs. EPA Method 1668A[4] is for low- and trace-level analysis of PCBs. It involves matrix-specific extraction, analyte-specific cleanup, and HRGC /HRMS analysis. This method provides very accurate determination of the WHO-designated dioxin-like PCBs and can be used to determine all 209 PCB congeners. Not all PCBs are determined individually with this method because some are determined as sets of coeluting congeners. The calibration range for PCBs based on a typical 10-g sample and 20-µL final sample volume is from 0.4 to 4,000 pg/g. PCBs also can be determined as specific congeners by GC/LRMS or as Aroclors[1] by GC/electron capture detection.

5.1.4 Reference Method Selection

Three EPA analytical methods for the quantification of dioxins and furans were available: Method 1613B, Method 8290, and Method 8280. Method 8280 is a LRMS method that does not have adequate sensitivity (i.e., the detection limits reported by the developers are less than that of the LRMS method). Methods 1613B and 8290 are HRMS methods with lower detection limits. Method 1613B includes more labeled internal standards than Method 8290, which affords more accurate congener quantification. Therefore, it was determined that Method 1613B best met the needs of the demonstration, and it was selected as the D/F reference method. Reference data of equal quality needed to be generated to determine the PCB contribution to the TEQ, since risk assessment is often based on TEQ values that are not class-specific. As such, the complementary HRMS method for PCB TEQ determinations, Method 1668A,[4] was selected as the reference method for PCBs. Total $TEQ_{D/F}$ concentrations were generated by Method 1613B, and total TEQ_{PCB} concentrations were generated by Method 1668A. These data were summed to derive a total TEQ value for each sample.

5.2 Characterization of Environmental Samples

All of the homogenized environmental samples were analyzed by the Battelle characterization laboratory to determine which would be included in the demonstration. The environmental samples were characterized for the 17 D/Fs by Method 1613B, the 12 WHO PCBs by LRMS-modified Method 1668A, and 18 target PAHs by the NOAA Status and Trends GC/Mass Spectrometry (MS) method.[7]

5.2.1 Dioxins and Furans

Four aliquots of homogenized material and one unhomogenized (i.e., "as received") aliquot were prepared and analyzed for seventeen 2,3,7,8-substituted dioxins and furans following procedures in EPA Method 1613B. The homogenized and unhomogenized aliquots were each approximately 200 g. Depending on the anticipated levels of dioxins from preliminary information received from each sampling location, approximately 1 to 10 g of material were taken for analysis from each aliquot, spiked with $^{13}C_{12}$-labeled internal standards, and extracted with methylene chloride using accelerated solvent extraction (ASE) techniques. One method blank and one laboratory control spike were processed with the batch of material from each site. The sample extracts were processed through various cleanup techniques, which included gel permeation chromatography or acid/base washes, as well as acid/base silica and carbon cleanup columns. As warranted, based on sample compositions, some samples were put through additional acid silica cleanup prior to the carbon column cleanup. Extracts were spiked with $^{13}C_{12}$-labeled recovery standards and concentrated to a final volume of 20 to 50 µL. Dilution and reanalysis of the extracts were performed if high levels of a particular congener were observed in the initial analysis; however, extracts were not rigorously evaluated to ensure that all peaks were below the peak area of the highest calibration standard.

Each extract was analyzed by HRGC/HRMS in the selected ion monitoring (SIM) mode at a resolution of 10,000 or greater. A DB-5 column was used for analysis of the seventeen 2,3,7,8-PCDD/F congeners. The instrument was calibrated for PCDD/F at levels specified in Method 1613B with one additional calibration standard at concentrations equivalent to one-half the level of Method 1613B's lowest calibration point. Using a DB5 column, 2,3,7,8-TCDF is not separated from other non-2,3,7,8-TCDF isomers. However, since the primary objective was to determine adequacy of homogenization and not congener quantification, it was determined that sufficient information on precision could be obtained

with the DB5 analysis of 2,3,7,8-TCDF and no second column confirmation of 2,3,7,8-TCDF was performed. PCDD/F data were reported as both concentration (pg/g dry) and TEQs (pg TEQ/g dry).

5.2.2 PCBs

One aliquot of material from each sampling location was prepared and analyzed for the 12 WHO-designated dioxin-like PCBs by GC/LRMS. The LRMS PCB analysis method is based on key components of the PCB congener analysis approach described in EPA Method 1668A and the PCB homologue approach described in EPA Method 680. Up to 30 g of sample were spiked with surrogates and extracted with methylene chloride using shaker table techniques. The mass of sample extracted was determined based on information supplied to the laboratory regarding possible contaminant concentrations. The extract was dried over anhydrous sodium sulfate and concentrated. Extracts were processed through alumina column cleanup, followed by high-performance liquid chromatography/gel permeation chromatography (HPLC/GPC). Additionally, sulfur was removed using activated granular copper. The post-HPLC extract was concentrated and fortified with recovery internal standards. Extracts were concentrated to a final volume between 500 microliters and 1 mL, depending on the anticipated concentration of PCBs in the sample, as reported by the sample providers. PCB congeners and PCB homologues were separated via capillary gas chromatography on a DB5-XLB column and identified and quantified using electron ionization MS. This method provides specific procedures for the identification and measurement of the selected PCBs in SIM mode.

5.2.3 PAHs

One aliquot of material from each sampling location was analyzed for PAHs. The 18 target PAHs included:

- naphthalene
- 2-methylnaphthalene
- 2-chloronaphthalene
- acenaphthylene
- acenaphthene
- fluorene
- phenanthrene
- anthracene
- fluoranthene
- pyrene
- benzo(a)anthracene
- chrysene
- benzo(b)fluoranthene
- benzo(k)fluoranthene
- benzo(a)pyrene
- indeno(1,2,3-cd)pyrene
- dibenzo(a,h)anthracene
- benzo(g,h,i)perylene.

The method for the identification and quantification of PAH in sediment and soil extracts by GC/MS was based on the NOAA Status and Trends method[7] and, therefore, certain criteria (i.e., initial calibrations and daily verifications) are different from those defined in traditional EPA methods 625 and 8270C. Up to 30 g of sample were spiked with surrogates and extracted using methylene chloride using shaker table techniques. The mass of sample extracted was determined based on information supplied to the characterization laboratory regarding possible contaminant concentrations. The extract was dried over anhydrous sodium sulfate and concentrated. The extract was processed through an alumina cleanup column followed by HPLC/GPC. The post-HPLC extract was concentrated and fortified with recovery internal standards. Extracts were concentrated between 500 µL and 1 mL, depending on the anticipated concentration of PCBs in the sample, as reported by the sample providers. PAHs were separated by capillary gas chromatography on a DB-5, 60-m column and were identified and quantified using electron impact MS. Extracts were analyzed in the SIM mode to achieve the lowest possible detection limits.

5.3 Reference Laboratory Selection

Based on a preliminary evaluation of performance and credibility, 10 laboratories were contacted and were sent a questionnaire geared toward understanding the capabilities of the laboratories, their experience with analyzing dioxin samples for EPA, and their ability to meet the needs of this demonstration. Two laboratories were selected for the next phase of the selection process and were sent three blind audit samples. Each laboratory went through a daylong audit that included a technical systems audit and a quality systems audit. At each laboratory, the audit consisted of a short opening conference; a full day of observation of laboratory procedures, records, interviews with laboratory staff; and a brief closing meeting. Auditors submitted followup questions to each laboratory to address gaps in the observations.

Criteria for final selection were based on the observations of the auditors, the performance on the audit samples, and cost. From this process, it was determined that AXYS Analytical Services (Sidney, British Columbia, Canada) would best meet the needs of this demonstration.

5.4 Reference Laboratory Sample Preparation and Analytical Methods

AXYS Analytical Services received all 209 samples on April 27, 2004. To report final data, AXYS submitted 14 D/F and 14 PCB data packages from June 11 to December 20, 2004. The following sections briefly describe the reference methods performed by AXYS.

5.4.1 Dioxin/Furan Analysis

All procedures were carried out according to protocols as described in AXYS Summary Method Doc MSU-018 Rev 2 18-Mar-2004 [AXYS detailed Standard Operating Procedure (SOP) MLA-017 Rev 9 May-2004], which is based on EPA Method 1613B. AXYS modifications to the method are summarized in the D/QAPP.[2] Briefly, samples were spiked with a suite of isotopically labeled surrogate standards prior to extraction, solvent extracted, and cleaned up through a series of chromatographic columns that included silica, Florisil, carbon/Celite, and alumina columns. The extract was concentrated and spiked with an isotopically labeled recovery (internal) standard. Analysis was performed using an HRMS coupled to an HRGC equipped with a DB-5 capillary chromatography column [60 meter (m), 0.25-mm internal diameter (i.d.), 0.1-µm film thickness]. A second column, DB-225 (30 m, 0.25-mm i.d., 0.15-µm film thickness), was used for confirmation of 2,3,7,8-TCDF identification. Samples that were known to contain extremely high levels of PCDD/F were extracted without the addition of the surrogate standard, split, then spiked with the isotopically labeled surrogate standard prior to cleanup. This approach allowed extraction of the method specified 10-g sample volume, and subsequent sufficient dilution that high level analytes were brought within the instrument calibrated linear range. While this approach induces some uncertainty because the actual recovery of analytes from the extraction process is unknown, it was decided by the demonstration panel that in general analyte recovery through the extraction procedures are known to be quite good and that the uncertainty introduced by this approach would be less than the uncertainty introduced by other approaches such as extracting a significantly smaller sample size.

5.4.2 PCB Analysis

The method was carried out in accordance with the protocols described in AXYS Summary Method Doc MSU-020 Rev 3 24-Mar-2004 (AXYS detailed SOP MLA-010 Rev 5 Sep-2003), which is based on EPA Method 1668A, with changes through August 20, 2003. AXYS modifications to the method are summarized in the D/QAPP. Briefly, samples were spiked with isotopically labeled surrogate standards, solvent extracted, and cleaned up on a series of chromatographic columns that included silica, Florisil, alumina, and carbon/Celite columns. The final extract was spiked with isotopically labeled recovery (internal) standards prior to instrumental analysis. The extract was analyzed by HRMS coupled to an HRGC equipped with a DB-1 chromatography column (30 m, 0.25-mm i.d., 0.25-µm film thickness). Because only the WHO-designated dioxin-like PCBs were being analyzed for this program and in order to better eliminate interferences, all samples were analyzed using the DB-1 column, which is an optional confirmatory column in Method 1668A rather than the standard SPB Octyl column. Samples that were known to contain extremely high levels of PCBs were extracted without the addition of the surrogate standard, split, then spiked with the isotopically labeled surrogate standard prior to cleanup. This approach allowed extraction of the method specified 10-g sample volume, and subsequent sufficient dilution that high level analytes were brought within the instrument calibrated linear range. While this approach induces some uncertainty because the actual recovery of analytes from the extraction process is unknown, it was decided by the demonstration panel that in general analyte recovery through the extraction procedures are known to be quite good and that the uncertainty introduced by this approach would be less than the uncertainty introduced by other approaches such as extracting a significantly smaller sample size.

5.4.3 TEQ Calculations

For the reference laboratory data, D/F and PCB congener concentrations were converted to TEQ and subsequently summed to determine total TEQ, using the TEFs established by WHO in 1998 (see Table 4-1).[5] Detection limits were reported as sample-specific

detection limits (SDLs). SDLs were determined from 2.5 times the noise in the chromatogram for D/F and 3.0 times the noise for PCBs, converted to an area, and then converted to a concentration using the same calculation procedure as for detected peaks. Any value that met all quantification criteria (> SDL and isotope ratio) were reported as a concentration. A "J" flag was applied to any reported value between the SDL and the lowest level calibration. The concentration of any detected congener that did not meet all quantification criteria (such as isotope ratio or peak shape) was reported but given a "K" flag to indicate estimated maximum possible concentration (EMPC).[8] TEQs were reported in two ways to cover the range of possible TEQ values:

(1) All nondetect and EMPC values were assigned a zero concentration in the TEQ calculation.

(2) Nondetects were assigned a concentration of one half the SDL. EMPCs were assigned a value equal to the EMPC.

In both cases, any total TEQ value that had 10% contribution or more from J-flagged or K-flagged data was flagged as J or K (or both) as appropriate.

TEQs were calculated both ways for all samples. For $TEQ_{D/F}$, 63% of the samples had the same TEQ value based on the two different calculation methods, and the average RPD was 8% (median = 0%). For TEQ_{PCB}, 65% of the samples had the same TEQ value based on the two different calculation methods, and the average RPD was 9% (median = 0%). Because overall there were little differences between the two calculation methods, TEQ values calculated by option #1 were used in comparison with the developer technologies (as presented in Appendix D). On a case-by-case basis, developer results were compared to TEQs calculated by option #2 above, but no significant differences in comparability results were observed so no additional data analysis results using these TEQ values were presented.

Chapter 6
Assessment of Reference Method Data Quality

Ensuring reference method data quality is of paramount importance to accurately assessing and evaluating each of the innovative technologies. To ensure that the reference method has generated accurate, defensible data, a quality systems/technical audit of the reference laboratory was performed during analysis of demonstration samples after the first batch of demonstration sample analyses was complete. The quality systems/technical audit evaluated implementation of the demonstration plan. In addition, a full data package was prepared by the reference laboratory for each sample batch for both dioxin and dioxin-like PCB analyses. Each data package was reviewed by both a QA specialist and technical personnel with expertise in the reference methods for agreement with the reference method as described in the demonstration plan. Any issues identified during the quality systems/technical audit and the data package reviews were addressed by the reference laboratory prior to acceptance of the data. In this section, the reference laboratory performance on the QC parameters is evaluated. In addition, the reference data were statistically evaluated for the demonstration primary objectives of accuracy and precision.

6.1 QA Audits

A quality systems/technical audit was conducted at the reference laboratory, AXYS Analytical Services, Ltd., by Battelle auditors on May 26, 2004, during the analysis of demonstration samples. The purpose of the audit was to verify AXYS compliance with its internal quality system and the D/QAPP.[2] The scope specifically included a review of dioxin and PCB congener sample processing, analysis, and data reduction; sample receipt, handling, and tracking; supporting laboratory systems; and followup to observations and findings identified during the independent laboratory assessment conducted by Battelle on February 11, 2004, prior to contract award. Checklists were prepared to guide the audit, which consisted of a review of laboratory records and documents, staff interviews, and direct observation.

The AXYS quality system is documented in a comprehensive QA/QC manual and detailed standard operating procedures (SOPs). No major problems or issues were noted during the audit. Two findings were identified, one related to a backlog of unfiled custody records and the other related to the need for performance criteria for the DB-1 column used for the analysis of PCB congeners by HRMS. Both issues were addressed satisfactorily by AXYS after the audit. One laboratory practice that required procedural modification was identified: the laboratory did not subject all QC samples to the most rigorous cleanup procedures that might be required for individual samples within a batch. The AXYS management team agreed that this procedure was incorrect. As corrective action, the QA manager provided written instructions regarding cleanup of the quality control samples to the staff, and the laboratory manager conducted follow up discussion with the staff. Other isolated issues noted by the auditors did not reflect systemic problems and were typical of analytical laboratories (e.g., occasional documentation lapses or an untrackable balance weight).

The audit confirmed that the laboratory procedures conformed to the SOPs and D/QAPP and that the quality system was implemented effectively. Samples were processed and analyzed according to the laboratory SOPs and D/QAPP using the Soxhlet-Dean Stark extraction method. No substantial deviations were noted. The audit verified the traceability of samples within the laboratory, as well as the traceability of standards, reagents, and solvents used in preparation, and that the purity and reliability of the latter materials were demonstrated through documented quality checks. In addition, the audit confirmed that analytical instruments and equipment were maintained and

calibrated according to manufacturers' specifications and laboratory SOPs. Analytical staff members were knowledgeable in their areas of expertise. QC samples were processed and analyzed with each batch of authentic samples as specified by the D/QAPP. QA/QC procedures were implemented effectively, and corrective action was taken to address specific QC failures. Data verification, reporting, and validation procedures were found to be rigorous and sufficient to ensure the accuracy of the reported data. The auditors concluded that AXYS is in compliance with the D/QAPP and its SOPs, and that the data generated at the laboratory are of sufficient and known quality to be used as a reference method for this project.

In addition, each data package was reviewed by both a QA specialist and technical personnel with expertise in the reference methods for agreement with the reference method as described in the demonstration plan. Checklists were prepared to guide the data package review. This review included an evaluation of data package documentation such as chain-of-custody (COC) and record completeness, adherence to method prescribed holding times and storage conditions, standard spiking concentrations, initial and continuing calibrations meeting established criteria, GC column performance, HRMS instrument resolution, method blanks, lab control spikes (ongoing precision and recovery samples), sample duplicates, internal standard recovery, transcription of raw data into the final data spreadsheets, calculation of TEQs, and data flag accuracy. Any issues identified during the data package reviews were addressed by the reference laboratory prior to acceptance of the data. All of the audit reports and responses are included in the DER.

6.2 QC Results

Each data package was reviewed for agreement with the reference method as described in the demonstration plan. This section summarizes the evaluation of the reference method quality control data.

6.2.1 Holding Times and Storage Conditions

All demonstration samples were stored frozen (< -10°C) upon receipt and were analyzed within the method holding time of one year.

6.2.2 Chain of Custody

All sample identifications were tracked from sample login to preparation of record sheets, to instrument analysis sheets, to the final report summary sheets and found to be consistent throughout. One COC with an incomplete signature and one discrepancy in date of receipt between the COC and sample login were identified during the Battelle audit and were corrected before the data packages with these affected items were accepted as final.

6.2.3 Standard Concentrations

The concentration of all calibration and spiking standards was verified.

6.2.4 Initial and Continuing Calibration

All initial calibrations met the criteria for response factor RSD and minimal signal-to-noise ratio requirements for the lowest calibration point.

Continuing calibrations were performed at the beginning and end of every 12-hour analysis period with one minor exception for D/F sample batch WG13551, which contained five samples from Environmental Site #1 (North Carolina) and 12 samples from Environmental Site #5 (Winona Post). On one analysis day, a high-level sample analyzed just prior to the ending calibration verification caused the verification to fail. In this instance, the verification was repeated just outside of the 12-hour period. The repeat calibration verification met the acceptance criteria and was considered to show acceptable instrument performance in the preceding analytical period; therefore, the data were accepted.

Continuing calibration results were within the criteria stated in Table 9-2 (D/F) and Table 9-4 (PCB) of the D/QAPP, with one exception. For PCB sample batch WG12108, which contained nine samples from Environmental Site #3 (Newark Bay) and 12 samples from Environmental Site #4 (Raritan Bay), isotopically labeled PCB 169 was above the acceptable range during one calibration verification on May 15, 2004. The acceptance range included in the D/QAPP is tighter than the acceptance range in Method 1668A Table 6. Because the result for labeled PCB 169 was within the Method 1668A acceptance limits, the data were accepted. The minimum signal-to-noise criteria for analytes in the calibration verification solution were always met.

6.2.5 Column Performance and Instrument Resolution

Column performance was checked at the beginning of each 12-hour analytical period and met method criteria.

Instrument resolution was documented at the beginning and end of each 12-hour period with one exception. In PCB sample batch WG13554, which contained five performance evaluation samples and 15 extract samples, on one analysis day (September 17, 2004), the ending resolution documentation was conducted at 12 hours and 54 minutes. However, as this resolution documentation met all criteria, it was considered representative of acceptable instrument performance during the analytical period, and the data were accepted.

6.2.6 Method Blanks

Method blanks were analyzed with each sample batch to verify that laboratory procedures did not introduce significant contamination. A summary of the method blank data is presented in Appendix C. There were many instances for both D/F and PCB data where analyte concentrations in the method blank exceeded the target criteria in the D/QAPP. Samples from this demonstration, which had very high D/F and PCB concentrations, contributed to the difficulty in achieving method blank criteria in spite of steps the reference laboratory took to minimize contamination (such as proofing the glassware before use in each analytical batch). In many instances, the concentrations of D/F and PCBs in the samples exceeded 20 times the concentrations in the blanks. For all instances, the sample results were unaffected because the method blank TEQ concentration was compared to the sample TEQ concentrations to ensure that background contamination did not significantly impact sample results.

6.2.7 Internal Standard Recovery

Internal standard recoveries were generally within the D/QAPP criteria. D/QAPP criteria were tighter than the standard EPA method criteria; in instances where internal standard recoveries were outside of the D/QAPP criteria, but within the standard EPA method criteria, results were accepted. In several instances, the dioxin cleanup standard recoveries were affected by interferences. As the cleanup standard is not used for quantification of native analytes, these data were accepted. Any samples affected by internal standard recoveries outside of the D/QAPP and outside of the EPA method criteria were evaluated for possible impact on total TEQ and for comparability with replicates processed during the program before being accepted.

6.2.8 Laboratory Control Spikes

One laboratory control spike (ongoing precision and recovery sample), which consisted of native analytes spiked into a reference matrix (sand), was processed with each analytical batch to assess accuracy. Recovery of spiked analytes was within the D/QAPP criteria in Table 9-2 for all analytes in all laboratory control spike samples.

6.2.9 Sample Batch Duplicates

A summary of the duplicate data is presented in Appendix C. One sample was prepared in duplicate in most sample batches; four batches were reported without a duplicate. Three of 14 dioxin sample batches and 5 of 14 PCB sample batches did not meet criteria of < 20% RPD between duplicates. Data where duplicates did not meet D/QAPP criteria were evaluated on an individual basis.

6.3 Evaluation of Primary Objective P1: Accuracy

Accuracy was assessed through the analysis of PE samples consisting of certified standard reference materials, certified spikes, and certified blanks. A summary of reference method percent recovery (R) values is presented in Table 6-1. The R values are presented for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ. The minimum, maximum, mean, and median R values are presented for each set of TEQ results. The reference method values were in best agreement with the certified values for the TEQ_{PCB} results, with a mean R value of 96%. The mean R values for $TEQ_{D/F}$ and total TEQ were 125% and 94%, respectively. The mean and median R values for the TEQ_{PCB} and total TEQ were identical. The mean and median R values for $TEQ_{D/F}$ were not similar and were largely influenced by the $TEQ_{D/F}$ recovery for ERA Aroclor of 324%. The ERA Aroclor-certified $TEQ_{D/F}$ values were based on TCDD and TCDF only, whereas the reference method $TEQ_{D/F}$ values were based on contributions from all 2,3,7,8-substituted D/F analytes. The R values presented in Table 6-2 indicate that the reference method reported data that were on average between 94 and 125% of the certified values of the PE samples. The effect of known interferences on

Table 6-1. Objective P1 Accuracy - Percent Recovery

PE Sample ID	PE Sample Description	% Recovery					
		TEQ_{PCB}		$TEQ_{D/F}$		Total TEQ	
1	Cambridge 5183	81		111		94	
2	LCG CRM-529	100		106		106	
3	Wellington WMS–01	93		106		105	
4	Cambridge 5184	120		106		118	
5	NIST 1944	102		91		93	
6	ERA TCDD 10	NA		79		79	
7	ERA TCDD 30	NA		77		77	
8	ERA PAH	NA		NA		NA	
9	ERA PCB 100	96		NA		95	
10	ERA PCB 10000	95		NA		95	
11	ERA Aroclor	82		324		83	
12	ERA Blank	NA		NA		NA	
All Performance Evaluation Samples		NUMBER	8	NUMBER	8	NUMBER	10
		MIN	81	MIN	77	MIN	77
		MAX	120	MAX	324	MAX	118
		MEDIAN	96	MEDIAN	106	MEDIAN	94
		MEAN	96	MEAN	125	MEAN	94

NA = not applicable; insufficient data were reported to determine R or the sample was not spiked with those analytes.

Table 6-2. Evaluation of Interferences

PE Material with Known Interference	Mean TEQ (pg/g)
ERA PAH	0.195 (D/F + PCB)
ERA PCB 100	0.073 (D/F)
ERA PCB 10000	0.220 (D/F)
ERA TCDD 10	0.025 (PCB)
ERA TCDD 30	0.036 (PCB)

reference method TEQs is listed in Table 6-3. D/F and PCB TEQs were not affected by PAH as evidenced through the analysis of ERA PAH standard reference material. D/F and PCB TEQs were not affected by each other as evidenced by spikes that contained only one set of analytes having negligible influence on the TEQ of the other analyte set.

6.4 Evaluation of Primary Objective P2: Precision

The 209 samples included in the demonstration consisted of replicates of 49 discrete samples. There were four replicates of each sample except for PE sample Cambridge 5183 (7 replicates), ERA blank reference material (8 replicates), Wellington WMS-01 standard reference material (7 replicates), and 0.5 pg/mL 2,3,7,8-TCDD extract (7 replicates). Reference method data were obtained for all 209 samples; however, data for $TEQ_{D/F}$ and total TEQ from samples Ref 197 (ERA PCB 100) and Ref 202 (LCG CRM-529) were omitted as outliers as it appeared that these two samples were switched during preparation after observing results of the replicates and evaluating the congener profiles of these two samples.

A summary of the reference method replicate RSD values is presented in Tables 6-3a and 6-3b. The RSD values are presented for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ in Table 6-3a, and a summary by sample type is presented in Table 6-3b, along with the minimum R value, the maximum R value, and the mean R value for each set of TEQ results and sample types. In terms of sample type, the reference method had the most precise

Table 6-3a. Objective P2 Precision - Relative Standard Deviation

Sample Type	Sample ID	RSD for TEQ_{PCB} (%)	RSD for $TEQ_{D/F}$ (%)	RSD for Total TEQ (%)
Environmental	Brunswick #1	8	6	6
	Brunswick #2	3	16	16
	Brunswick #3	5	8	8
	Midland #1	4	9	9
	Midland #2	10	6	6
	Midland #3	4	6	6
	Midland #4	77	9	10
	NC PCB Site #1	21	15	20
	NC PCB Site #2	21	2	21
	NC PCB Site #3	25	12	24
	Newark Bay #1	7	28	25
	Newark Bay #2	2	22	20
	Newark Bay #3	6	6	6
	Newark Bay #4	1	12	11
	Raritan Bay #1	6	5	4
	Raritan Bay #2	3	2	1
	Raritan Bay #3	3	5	4
	Saginaw River #1	8	25	23
	Saginaw River #2	7	19	18
	Saginaw River #3	60	19	19
	Solutia #1	36	13	13
	Solutia #2	4	7	7
	Solutia #3	11	5	5
	Titta. River Soil #1	7	6	5
	Titta. River Soil #2	9	10	10
	Titta. River Soil #3	12	26	26
	Titta. River Sed #1	19	27	26
	Titta. River Sed #2	14	37	37
	Titta. River Sed #3	13	9	8
	Winona Post #1	13	2	2
	Winona Post #2	4	9	9
	Winona Post #3	9	4	4
Extract	Envir Extract #1	71	50	50
	Envir Extract #2	83	2	2
	Spike #1	119	6	9
	Spike #2	1	5	3
	Spike #3	4	13	4
Performance Evaluation	Cambridge 5183	7	19	9
	Cambridge 5184	3	4	2
	ERA Aroclor	44	6	43
	ERA Blank	62	65	61
	ERA PAH	83	27	30
	ERA PCB 100	4	65 [a]	3
	ERA PCB 10000	7	91	7
	ERA TCDD 10	60	5	5
	ERA TCDD 30	39	6	6
	LCG CRM-529	14	2[a]	1
	NIST 1944	4	9	7
	Wellington WMS-01	5	3	3

[a] Does not include sample excluded due to sample preparation error.

Table 6-3b. Objective P2 Precision - Relative Standard Deviation (By Sample Type)

Sample Type	RSD for TEQ_{PCB} (%)					RSD for $TEQ_{D/F}$ (%)					RSD for Total TEQ (%)				
	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN
Environmental	32	1	77	8	13	32	2	37	9	12	32	1	37	10	13
Extract	5	1	119	71	56	5	2	50	6	15	5	2	50	4	14
PE	12	3	83	11	28	12	2	91	7	25	12	1	61	7	15
Overall	49	1	119	8	21	49	2	91	9	16	49	1	61	8	13

data for the environmental sample $TEQ_{D/F}$ results, with a mean RSD value of 12%. This was followed closely by environmental sample TEQ_{PCB} and total TEQ results, which both had mean RSDs of 13%. In terms of TEQ values, the reference method had the most precise data for the total TEQ values, with a mean overall RSD of 13%. Overall RSD values ranged from 1% to 119%. Precision was significantly worse for certified blanks and blank samples (e.g., samples that contained spikes of only one analyte set and were blank for the other analytes) as might be expected due to the very low levels detected in these samples.

6.5 Comparability to Characterization Data

To assess comparability, reference laboratory D/F data for environmental samples were plotted against the characterization data that was generated by Battelle prior to the demonstration. Characterization data were obtained as part of the process to verify homogenization of candidate soil and sediment samples as described in Chapter 5 and reported in Table 4-5. It should be noted that second column confirmations of 2,3,7,8-TCDF results were not performed during characterization analyses; therefore, characterization TEQs are biased high for samples where a large concentrations of non-2,3,7,8-TCDF coeluted with 2,3,7,8-TCDF on the DB-5 column. Characterization samples also were not rigorously evaluated to ensure that high concentration extracts were diluted sufficiently so that all peak areas were less than the peak areas of the highest calibration standard. In spite of these differences between reference and characterization analyses, the results had fairly high correlation ($R^2 = 0.9899$) as demonstrated in Figure 6-1.

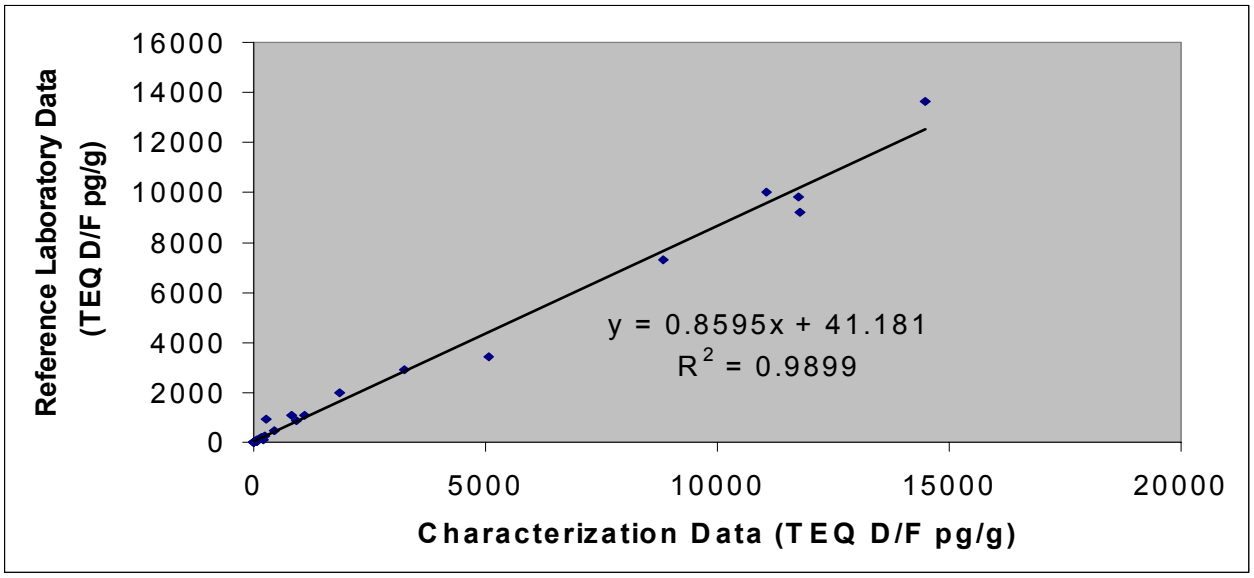


Figure 6-1. Comparison of reference laboratory and characterization D/F data for environmental samples.

6.6 Performance Summary

This section provides a performance summary of the reference method by summarizing the evaluation of the applicable primary objectives of this demonstration (accuracy, precision, and cost) in Table 6-4. A total of 209 samples was analyzed for seventeen 2,3,7,8-substituted D/F and 12 PCBs over an eight-month time frame (April 27 to December 20, 2004). Valid results were obtained for all 209 PCB analyses, while 207 valid results were obtained for D/F. The D/F and total TEQ results for samples Ref 197 (ERA PCB 100) and Ref 202 (LCG CRM-529) were omitted as outliers because it appeared that these two samples were switched during preparation after observing results of the replicates and evaluating the congener profiles of these two samples. The demonstration sample set provided particular challenges to the reference laboratory in that there was a considerable range of sample concentrations for D/F and PCB. This caused some difficulty in striving for low MDLs in the presence of high-level samples. The range of concentrations in the demonstration sample set also required the laboratory to modify standard procedures, which contributed to increased cost and turnaround time delay. For example, an automated sample cleanup system could not be used due to carryover from high-level samples; instead, more labor-intensive manual cleanup procedures were used; glassware required extra cleaning and proofing before being reused; cleanup columns sometimes became overloaded from interferences and high-level samples, causing low recoveries so that samples had to be re-extracted or cleanup fractions had to be analyzed for the lost analytes; and method blanks often showed trace levels of contamination, triggering the repeat of low-level samples.

Because the reference method was not to be altered significantly for this demonstration, the reference laboratory was limited in its ability to adapt the trace-level analysis to higher level samples. In spite of these challenges, the quality of the data generated met the project goals. The main effect of the difficulties associated with these samples was on schedule and cost.

Table 6-4. Reference Method Performance Summary - Primary Objectives

Objective	Performance			
	Statistic	TEQ_{PCB}	$TEQ_{D/F}$	Total TEQ
P1: Accuracy	Number of data points	8	8	10
	Median Recovery (%)	96	106	94
	Mean Recovery (%)	96	125	94
P2: Precision	Number of data points	49	49	49
	Median RSD (%)	8	9	8
	Mean RSD (%)	21	16	13
P7: Cost	209 samples were analyzed for 17 D/F and 12 PCBs. Total cost was $398,029. D/F cost was $213,580 ($1,022 per sample) and PCB cost was $184,449 ($883 per sample).			

Chapter 7
Performance of Xenobiotic Detection Systems, Inc., CALUX® by XDS

7.1 Evaluation of CALUX® by XDS Performance

The Xenobiotic Detection Systems, Inc. CALUX® by XDS is an aryl hydrocarbon-receptor bioassay that individually reports the TEQ of D/Fs and PCBs in the sample. When comparing the CALUX® by XDS results with HRMS TEQ results from the certified samples and the reference methods, the reader should keep in mind the limitations of the TEQ approach described in Section 4.2. Note that it is possible that Ah-receptor binding compounds that are being measured during the XDS analysis are not all accounted for in the reference laboratory TEQ result and that the 1998 WHO TEFs used to generate the reference laboratory TEQs may differ from the assay Ah-receptor binding affinity for certain analytes. Therefore, the technology should not be viewed as producing an equivalent measurement value to HRMS TEQ values for all samples. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint.

The following sections describe the performance of CALUX® by XDS, according to the primary objectives for this demonstration. The developer and reference laboratory data are presented in Appendix D. The statistical methods used to evaluate the primary objectives are described in Section 4.7. Detailed data evaluation records can be found in the DER.

7.1.1 Evaluation of Primary Objective P1: Accuracy

A summary of the CALUX® by XDS percent recovery (R) values is presented in Table 7-1. The description of how R values were calculated is presented in Section 4.7.1. The R values are presented for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ. The minimum, maximum, mean, and median R values are presented for each set of TEQ results. The CALUX® by XDS values were in best agreement with the certified values for the total TEQ results, with a mean R value of 217%. The mean R value for the TEQ_{PCB} and $TEQ_{D/F}$ results were 548% and 514%, respectively. The R values presented in Table 7-1 indicate that the CALUX® by XDS generally reported $TEQ_{D/F}$ and total TEQ data that were biased high relative to the certified values of the PE samples, and TEQ_{PCB} data that were generally biased low. Exceptions to this were the total TEQ R values for the PCB-only spiked PE samples and the Aroclor-spiked PE sample which also contained a low-level D/F spike. As shown in Appendix D, the PCB results reported by XDS for these samples were considerably lower than the certified values, causing the total TEQ results to also be low.

7.1.2 Evaluation of Primary Objective P2: Precision

A summary of the CALUX® by XDS RSD values is presented in Tables 7-2a and 7-2b. The description of how RSD values were calculated is presented in Section 4.7.2. The RSD values are presented for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ in Table 7-2a, and a summary by sample type is presented in Table 7-2b, along with the minimum R value, the maximum R value, and the mean R value for each set of TEQ results and sample types. Low RSD values (< 20 %) indicate high precision. In terms of sample type, the CALUX® by XDS values had the most precise data for the PE $TEQ_{D/F}$ results, with a mean RSD value of 34%. In terms of TEQ values, the CALUX® by XDS values had the most precise data for the $TEQ_{D/F}$ values, with an overall RSD of 41%. Overall RSD values ranged from 2% to 199%.

Table 7-1. Objective P1 Accuracy - Percent Recovery

PE Sample ID	PE Sample Description	% Recovery					
		TEQ_{PCB}		$TEQ_{D/F}$		Total TEQ	
1	Cambridge 5183	1,487		614		868	
2	LCG CRM-529	38		239		226	
3	Wellington WMS–01	1,736		332		392	
4	Cambridge 5184	3		538		85	
5	NIST 1944	12		282		243	
6	ERA TCDD 10	NA		148		160	
7	ERA TCDD 30	NA		120		121	
8	ERA PAH	NA		NA		NA	
9	ERA PCB 100	NA		NA		45	
10	ERA PCB 10000	3		NA		15	
11	ERA Aroclor	NA		1,842		17	
12	ERA Blank	NA		NA		NA	
All Performance Evaluation Samples		NUMBER	6	NUMBER	8	NUMBER	10
		MIN	3	MIN	120	MIN	15
		MAX	1,736	MAX	1,842	MAX	868
		MEDIAN	25	MEDIAN	307	MEDIAN	141
		MEAN	548	MEAN	514	MEAN	217

NA = not applicable; insufficient data were reported to determine R or the sample was not spiked with those analytes

[a] Three or four replicate results were used to calculate the RSD values.

Table 7-2a. Objective P2 Precision - Relative Standard Deviation

Sample Type	Sample ID	Relative Standard Deviation (% RSD)[a]		
		TEQ_{PCB}	$TEQ_{D/F}$	Total TEQ
Environmental	Brunswick #1	NA	82	83
	Brunswick #2	NA	34	43
	Brunswick #3	83	52	52
	Midland #1	96	20	24
	Midland #2	NA	28	28
	Midland #3	113	31	32
	Midland #4	NA	23	24
	NC Site #1	51	11	20
	NC Site #2	31	62	58
	NC Site #3	32	23	21
	Newark Bay #1	NA	32	31
	Newark Bay #2	NA	62	61
	Newark Bay #3	NA	37	37
	Newark Bay #4	NA	50	50
	Raritan Bay #1	NA	18	18
	Raritan Bay #2	NA	21	13
	Raritan Bay #3	NA	23	23
	Saginaw River #1	151	23	22
	Saginaw River #2	146	32	32
	Saginaw River #3	NA	2	3
	Solutia #1	NA	24	24
	Solutia #2	165	84	83
	Solutia #3	189	56	64
	Titta. River Soil #1	84	46	45
	Titta. River Soil #2	194	16	25
	Titta. River Soil #3	46	124	124
	Titta. River Sed #1	NA	85	85

Sample Type	Sample ID	Relative Standard Deviation (% RSD)[a]		
		TEQ_{PCB}	$TEQ_{D/F}$	Total TEQ
	Brunswick #1	NA	82	83
	Titta. River Sed #2	NA	57	57
	Titta. River Sed #3	NA	42	42
	Winona Post #1	NA	42	42
	Winona Post #2	114	81	82
	Winona Post #3	NA	76	76
Extracts	Envir Extract #1	64	94	92
	Envir Extract #2	NA	9	9
	Spike #1	NA	35	60
	Spike #2	NA	18	14
	Spike #3	NA	35	69
Performance Evaluation	Cambridge 5183	199	24	165
	Cambridge 5184	162	20	22
	ERA Aroclor	NA	31	125
	ERA Blank	99	NA	117
	ERA PAH	NA	NA	140
	ERA PCB 100	NA	76	143
	ERA PCB 10000	31	98	76
	ERA TCDD 10	NA	31	20
	ERA TCDD 30	NA	16	18
	LCG CRM-529	26	3	3
	NIST 1944	67	21	22
	Wellington WMS-01	163	23	74

NA = not applicable (i.e., one or more of the replicates were reported as a nondetect value).

[a] Three or four replicate results were used to calculate the RSD values.

Table 7-2b. Objective P2 Precision - Relative Standard Deviation (By Sample Type)

Sample Type	Relative Standard Deviation (% RSD)														
	TEQ_{PCB}					$TEQ_{D/F}$					Total TEQ				
	No.	MIN	MAX	MEAN	MED	No.	MIN	MAX	MEAN	MED	No.	MIN	MAX	MEAN	MED
Env	14	31	194	107	104	32	2	124	44	35	32	3	124	44	39
Ex	1	64	64	64	64	5	9	94	38	35	5	9	92	49	60
PE	7	26	199	107	99	10	3	98	34	23	12	3	165	77	75
All	22	26	199	105	97	47	2	124	41	32	49	3	165	53	42

7.1.3 Evaluation of Primary Objective P3: Comparability

The description of the statistical analyses used in the comparability evaluations are described in Section 4.7.3. In Table 7-3, the comparability of the XDS and reference laboratory data was assessed by calculating RPD values for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ is summarized. Table 7-3 provides an overall assessment of the RPD values that is reported by TEQ value and sample type. The XDS values agreed best with the reference laboratory D/F measurements for extract samples, with a median RPD value of -8%. The median RPD values for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ were -17%, -102%, and -92%, with minimum and maximum values around -200% and +200%, respectively. This evaluation indicates that the XDS results were generally higher than the reference laboratory (as evidenced by all median values being negative) and that the TEQ_{PCB} results were reported most consistently with the reference laboratory results. RPD values between positive and negative 25% indicate good agreement between the reference laboratory and developer values. Of the TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ values, five (5%), seventeen (9%), and nineteen (11%) of the samples, respectively, had RPD values between positive and negative 25%.

Comparability was also assessed using the interval approach discussed in Section 4.7.3. The agreement

Table 7-3. Objective P3 Comparability - RPD Summary Statistics

Sample Type	TEQ_{PCB} RPD (%)				$TEQ_{D/F}$ RPD (%)				TOTAL TEQ RPD (%)			
	N	MIN	MAX	MEDIAN	N	MIN	MAX	MEDIAN	N	MIN	MAX	MEDIAN
Environmental	71	-200	175	-91	127	-198	196	-105	127	-188	186	-95
Extract	9	-190	166	49	16	-136	74	-8	12	-136	98	-98
PE	25	-195	200	115	37	-160	-21	-107	29	-191	183	-85
Overall	105	-200	200	-17	180	-198	196	-102	168	-191	186	-92

when sorting the developer and reference laboratory results for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ data into four intervals ($\leq$ 50 pg/g, 50-500 pg/g, 500 to 5,000 pg/g, and $\geq$ 5,000 pg/g) is described in Table 7-4. The agreement between the developer and reference laboratory was 82% for TEQ_{PCB}, 69% for $TEQ_{D/F}$, and 72% for total TEQ. Interval reporting addresses the question whether a value reported by the technology would result in the same decision of what to do next with the sample if it was analyzed by the reference method. This interval assessment table indicates that from 18 to 31% of the time, the XDS analysis would have resulted in a different decision about the sample than if it was analyzed by the reference laboratory, based on the TEQs determined for this demonstration and the concentrations chosen for the interval.

The ERA blank samples contained levels of D/Fs and PCBs that were below the reporting limits of the developer technologies (see Table 4-4 certified values: 0.046 pg/g $TEQ_{D/F}$ and 0.01 pg/g TEQ_{PCB}). The XDS-reported concentrations were compared with the reference laboratory reported data for these samples in Table 7-5. XDS reported 6 of the 8 TEQ_{PCB} values as detections (ranging from 0.88 to 13.72 pg/g), so only two results were reported as nondetects and agreed with the reference laboratory results. For $TEQ_{D/F}$, only two of the results were reported as detections (0.75 pg/g and 13.74 pg/g), so six of eight results agreed with the reference laboratory's reporting of blank samples. It should be noted that the reference laboratory data presented in Table 7-5 were calculated with nondetect values assigned a zero concentration. When applying the TEQ calculation method of assigning nondetects with a concentration of one-half the SDL, the reference data increased, but the conclusions regarding agreement with the developer data remain the same.

7.1.4 Evaluation of Primary Objective P4: Estimated Method Detection Limit

It should be noted that these detection limit calculations did not strictly follow the definition as presented in the *Code of Federal Regulations* (i.e., t-value with 6 degrees of freedom). Since detections were not reported for all seven replicate samples, the degrees of freedom and statistical power of the analysis were reduced accordingly. The only approach that led to the use of the definitional calculation with 6 degrees of freedom required special treatment of the non-detect values (i.e., assigning values that were one-half or equal to the nondetect value). However, these calculations are provided as EMDLs to give the reader a sense of the detection capabilities of the technology.

The EMDL of the CALUX® by XDS was determined using Extract Spike #1. Seven samples were prepared in toluene spiked with 0.5 pg/mL of 2,3,7,8-TCDD only. Two other PE samples, Cambridge 5183 and Wellington WMS-01, were included in the demonstration in replicates of seven so that these samples could potentially be used for the EMDL calculation. These samples were not included in the EMDL evaluation, since the D/F and PCB levels were considerably higher than the detection capabilities of the CALUX® by XDS. Since 2,3,7,8-TCDD was the only congener spiked in Extract Spike #1, only an EMDL for $TEQ_{D/F}$ could be determined. As shown in Table 7-6, because some of the results for the samples were nondetects, the $TEQ_{D/F}$ EMDL was calculated in three ways: by setting nondetect values to zero, by setting nondetect values to half of the reporting limit value, and by setting nondetect values to the reporting limit value itself. For the seven Extract Spike #1 samples, XDS reported three as

Table 7-4. Objective P3 - Comparability Using An Interval Assessment

Agreement	TEQ_{PCB}	$TEQ_{D/F}$	Total TEQ
Number Agree	160	142	146
% Agree	82	69	72
Number Disagree	35	65	57
% Disagree	18	31	28

Table 7-5. Objective P3 - Comparability for Blank Samples

	TEQ_{PCB}			$TEQ_{D/F}$		
Rep	XDS (pg/g)	Ref Lab[a] (pg/g)	Agree?	XDS (pg/g)	Ref Lab[a] (pg/g)	Agree?
1	13.72	J0.0243[b]	No	ND <0.45	J0.0942	Yes
2	0.88	0.00385	No	ND <0.23	J0.0728	Yes
3	1.02	0.00277	No	ND <0.45	J0.237	Yes
4	6.87	J0.042	No	ND <0.45	J0.307	Yes
5	ND <1.26	J0.0229	Yes	ND <0.45	J0.113	Yes
6	1.91	J0.0191	No	ND <0.45	J0.0524	Yes
7	ND <0.50	J0.0325	Yes	0.75	J0.211	No
8	5.67	J0.0225	No	13.74	J0.0692	No
% agreement	25% (2 of 8)			75% (6 of 8)		

[a] All nondetect and EMPC values were assigned a zero concentration for the reference laboratory TEQ calculation.
[b] J flag was applied to any reported value between the SDL and the lowest level calibration.
ND = nondetect.

Table 7-6. Objective P4 - Estimated Method Detection Limit

	Extract Spike #1		
Statistic	Nondetect values set to zero	Nondetect values set to ½ value	Nondetect values set to reported value
Degrees of Freedom	3	6	6
SD (pg/g $TEQ_{D/F}$)	0.136	0.198	0.170
EMDL (pg/g $TEQ_{D/F}$)	0.62	0.63	0.53

nondetects (<0.13 pg/g TEQ). While the number of degrees of freedom ranged from 3 to 6 because of the nondetect values, the EMDLs for all three calculations were very similar (0.62 pg/g TEQ, 0.63 pg/g TEQ, and 0.53 pg/g TEQ). The detection limit reported by XDS in the demonstration plan was 0.3 pg/g TEQ.

7.1.5 Evaluation of Primary Objective P5: False Positive/False Negative Results

The description of false positive/false negative calculations is presented in Section 4.7.5. The summary of false positive/false negative results is presented in Table 7-7.

Table 7-7. Objective P5 - False Positive/False Negative Results

Rate	TEQ_{PCB}		$TEQ_{D/F}$		Total TEQ	
	1 pg/g	50 pg/g	1 pg/g	50 pg/g	1 pg/g	50 pg/g
False Positive	15% (29 of 194)	9% (18 of 194)	6% (12 of 207)	10% (20 of 207)	4% (8 of 207)	6% (12 of 207)
False Negative	23% (45 of 194)	6% (11 of 194)	0% (0 of 207)	0.5% (1 of 207)	1% (2 of 207)	0% (0 of 207)

The technology had a fairly high rate of false positive and false negative results around 1 pg/g TEQ_{PCB} (15% and 23%, respectively), but it had significantly fewer false positives and false negatives for total TEQ (4% and 1%, respectively) and $TEQ_{D/F}$ (6% and 0%, respectively). When the XDS results were compared to the reference laboratory for values around 50 pg/g TEQ, the false positive and false negative rates for all TEQ types were 10% or below.

These data suggest that the XDS technology could be an effective tool to screen samples as being above or below 1 pg/g TEQ for $TEQ_{D/F}$ and total TEQ, and that it could be effective for all three types of TEQ values to determine results above or below 50 pg/g TEQ.

7.1.6 Evaluation of Primary Objective P6: Matrix Effects

Six types of potential matrix effects were investigated: (1) sample analysis location (field vs. laboratory), (2) matrix type (soil vs. sediment vs. extract), (3) PAH concentration, (4) sample type (PE vs. environmental vs. extract) (5) environmental site, and (6) known interferences. A summary of the matrix effects is provided in the bullets below, followed by a detailed discussion:

- Measurement location: 21% statistically different
- Matrix type: none
- Sample type: slight for total TEQ
- PAH concentration: none
- Environmental site: none
- Known inteferences: slight

A one-way ANOVA was performed on samples that had at least one detected replicate analyzed in the field and in the laboratory to determine if performance was affected by the samples being analyzed in the field. A p-value less than 0.05 in Table 7-8 indicates that the mean of samples analyzed in the field was significantly different from the mean of those analyzed in the laboratory. Three of six TEQ_{PCB} measurements, one of 15 $TEQ_{D/F}$ measurements, and four of 18 total TEQ measurements showed statistically significant location effects. The majority (50%) of the TEQ_{PCB} values showed a significant difference, but only six sets had data that could be evaluated. Overall, 21% of the samples tested showed a statistically significant difference by sample analysis location, and of these samples, generally XDS reported the laboratory result more comparably to the reference laboratory result location. In Table 7-9, precision summary values are presented by matrix type. A one-way ANOVA model was used to test the effect of soil vs. sediment vs. extract on RSD. These tests showed no significant effect on RSD for TEQ_{PCB}, $TEQ_{D/F}$, or total TEQ. In Table 7-10, precision summary values are presented by PAH concentrations for environmental samples only. A one-way ANOVA model was used to test the effect of PAH concentration on RSD. These tests showed no significant effect on RSD for TEQ_{PCB}, $TEQ_{D/F}$, or total TEQ. The summary of RSD values segregated by sample type is presented in Table 7-2b. A one-way ANOVA model was used to test the effect of sample type (PE vs. environmental vs. extract) on RSD. These tests showed no significant effect on RSD for TEQ_{PCB} or $TEQ_{D/F,}$ but it did show a slight effect ($p = 0.0471$) for total TEQ. Based on the comparability results (RPD), XDS's results were not more or less comparable for one particular environmental site, suggesting that matrix effects were not dependent on environmental sites.

Table 7-8. Objective P6 - Matrix Effects Using Descriptive Statistics and ANOVA Results Comparing Replicate Analysis Conducted During Field Demonstration and in the Laboratory

Sample Type	Sample	Location	TEQ_{PCB}			$TEQ_{D/F}$			Total TEQ		
			N	Mean (SD) (pg/g)	p-Value Comparing Field to Laboratory	N	Mean (SD) (pg/g)	p-Value Comparing Field to Laboratory	N	Mean (SD) (pg/g)	p-Value Comparing Field to Laboratory
Environmental	Brunswick #1	field	0	NA[a]	--[b]	1	678.4	0.8689	1	678.4	0.8626
		lab	2	19.9 (24.9)		3	852.2 (805.2)		3	865.5 (826.1)	
	Midland #3	field	1	2.6	0.7119	1	569.0	0.7595	1	571.5	0.7605
		lab	2	7.7 (8.6)		3	664.4 (235.9)		3	669.5 (243.3)	
	NC PCB Site #2	field	1	> 39302.4	--	1	> 47,853.3	--	1	8,7155.7	**0.0080**
		lab	3	82,944.0 (6,460.9)		3	73,5906.8 (5,7349.2)		3	818,850.8 (5,7033.9)	
	Saginaw River #1	field	1	117.4	**0.0020**[c]	1	2,340.0	0.2667	1	2,457.4	0.3051
		lab	3	8.8 (4.2)		3	3,406.8 (605.7)		3	3,415.6 (607.4)	
	Saginaw River #3	field	0	NA	--	1	551.3	0.3371	1	551.3	0.3384
		lab	1	1.6		3	569.6 (12.7)		3	570.1 (13.1)	
	Saginaw River #4	field	0	NA	--	1	83.4	0.1333	1	83.4	0.1333
		lab	0	NA		3	39.4 (15.5)		3	39.4 (15.5)	
	Solutia #1	field	1	2.4	--	1	293.2	**0.0341**	1	295.6	**0.0279**
		lab	1	3.1		3	193.1 (16.4)		3	194.2 (15.0)	
	Titta. River Soil #2	field	1	819.4	**0.0000**	1	1668.3	0.9502	1	2,487.6	0.1827
		lab	3	6.3 (3.8)		3	1,695.8 (338.2)		3	1,702.0 (339.1)	
	Titta. River Sed #2	field	1	1.9	--	1	303.4	0.7388	1	305.3	0.7391
		lab	1	6.3		3	419.9 (263.6)		3	422.0 (264.4)	
	Winona Post #3	field	1	172.7	--	1	15502.3	0.3540	1	15,675.0	0.3555
		lab	1	99.2		3	73,561.7 (42,016.4)		3	73,594.8 (42073.6)	

Sample Type	Sample	Location	TEQ_{PCB}			$TEQ_{D/F}$			Total TEQ		
			N	Mean (SD) (pg/g)	p-Value Comparing Field to Laboratory	N	Mean (SD) (pg/g)	p-Value Comparing Field to Laboratory	N	Mean (SD) (pg/g)	p-Value Comparing Field to Laboratory
PE	Cambridge 5183	field	1	3.2	0.6612	1	28.5	0.4423	1	31.7	0.7360
		lab	4	92.5 (164.7)		6	23.2 (5.9)		6	84.8 (138.0)	
	Cambridge 5184	field	0	NA	--	1	807.5	0.6047	1	807.5	0.5730
		lab	3	28.3 (45.9)		3	956.1 (211.5)		3	984.3 (229.3)	
	ERA Aroclor	field	1	1,690.2	--	2	168.9 (2.1)	0.3857	2	1,014.0 (1,197.3)	0.4870
		lab	1	110.7		2	236.4 (86.7)		2	291.8 (165.0)	
	ERA Blank	field	1	5.7	0.9023	1	13.7	--	1	19.4	**0.0427**
		lab	5	4.9 (5.5)		1	0.8		6	4.2 (5.2)	
	ERA PAH	field	0	NA	--	1	2.9	--	1	2.9	0.7050
		lab	1	27.3		1	1.2		2	14.3 (18.5)	
	ERA TCDD 30	field	1	1.9	--	1	45.7	0.3704	1	47.5	0.2943
		lab	0	NA		3	37.6 (6.1)		3	37.6 (6.1)	
	LCG CRM-529	field	0	NA	--	1	1,5684.8	0.9727	1	1,5684.8	0.8128
		lab	3	163.1 (42.1)		3	1,5713.6 (644.9)		3	1,5876.7 (616.4)	
	Wellington WMS-01	field	1	524.5	**0.0043**	1	228.6	0.6475	1	753.1	**0.0001**
		lab	2	11.2 (2.8)		6	202.3 (50.0)		6	206.1 (45.9)	

[a] NA = not available; data reported as < or > (value).

[b] p-Value could not be determined because either the field or lab value was NA.

[c] **Bold** indicates field measurement statistically different from the laboratory measurement at the $p<0.05$ significance level.

Table 7-9. Objective P6 - Matrix Effects Using RSD as a Description of Precision by Soil, Sediment, and Extract

Matrix Type	RSD for TEQ_{PCB} (%)					RSD for $TEQ_{D/F}$ (%)					RSD for Total TEQ (%)				
	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN
Soil	16	26	199	97	102	24	3	124	31	44	26	3	165	43	61
Sediment	5	67	163	146	122	18	2	85	33	39	18	3	85	39	41
Extract	1	64	64	64	64	5	9	94	35	38	5	9	92	60	49
Overall	22	26	199	97	105	47	2	124	32	41	49	3	165	42	53

Table 7-10. Objective P6 - Matrix Effects Using RSD as a Description of Precision by PAH Concentration Levels (Environmental Samples Only)

PAH Concentration Level (ng/g)	RSD for TEQ_{PCB} (%)					RSD for $TEQ_{D/F}$ (%)					RSD for Total TEQ (%)				
	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN
> 100,000	3	31	83	32	49	3	23	62	52	46	3	21	58	52	44
10,000-100,000	1	114	114	114	114	4	42	82	79	70	4	42	83	79	71
1,000-10,000	7	51	189	146	130	16	11	84	31	35	16	13	83	31	36
< 1,000	3	46	194	84	108	9	2	124	42	47	9	3	124	42	47
Overall (Environmental Samples Only)	14	31	194	104	107	32	2	124	35	44	32	3	124	39	44

The effect of known interferences was also assessed by evaluating the results of PE materials that contained one type of contaminant (D/F, PCBs, or PAHs) but not another. Table 7-11 summarizes the detection of analytes not spiked in the PE samples, along with the percent recovery values (from Table 7-1) for the spiked analytes. For the ERA PAH sample that contained no spike D/Fs or PCBs, XDS reported a mean total TEQ value of 10.5 pg/g. The PCB-only spiked samples were reported with D/F concentrations that were 10% of the PCB certified concentration. XDS reported only one sample as a slight PCB detection for the D/F-only spiked PE samples.

7.1.7 Evaluation of Primary Objective P7: Technology Costs

Evaluation of this objective is fully described in Chapter 8, Economic Analysis.

Table 7-11. Objective P6 - Matrix Effects of Known Interferences Using PE Materials

PE Sample	% Recovery for Spiked Analytes [a]	Mean TEQ (pg/g) Reported by XDS for Analytes that were not Spiked in the PE Sample
ERA PAH	NA[b]	10.5 (total)
ERA PCB 100	NA	1.1 (D/F)
ERA PCB 10,000	3% (PCB)	125 (D/F)
ERA TCDD 10	148% (D/F)	5.35 (PCB)[c]
ERA TCDD 30	120% (D/F)	1.86 (PCB)[c]

[a] Percent recovery values taken from Table 7-1.

[b] NA = not applicable because R value could not be calculated.

[c] Three replicates were reported as nondetects.

7.2 Observer Report: Evaluation of Secondary Objectives

The CALUX® by XDS technology is based on a genetically engineered cell line containing the firefly luciferase gene under transactivational control of the

Ah receptor. This cell line is used to detect and quantify Ah-receptor agonists in a sample extract. Increasing Ah receptor activity in a sample extract will cause increasing expression of firefly luciferase, which is detected as light emission from the activated cells. XDS has developed and patented proprietary cleanup procedures to separate PCBs from PCDD/Fs in a sample extract prior to analysis and can, therefore, give results for PCBs and PCDD/Fs separately or combined. This technology may be used to screen samples or to provide a quantitative analysis. Currently, samples may be sent to XDS for analysis or the technology may be licensed. Steps observed during the demonstration included transferring extract samples, extracting soil samples, processing extracts through cleanup columns, dosing cells, and final read-out of results. Samples were prepared as outlined in the demonstration plan with the exception that 2 g of each solid sample were extracted instead of 1 g.

7.2.1 Evaluation of Secondary Objective S1: Skill Level of Operator

In the field demonstration, this technology was operated solely by Dr. John Gordon. Dr. Gordon is the research director at XDS and has a Ph.D. in biochemical genetics with over seven years of experience in biochemistry, cell culture, molecular biology, and chemistry. A second person (a nonscientist) was available to assist as necessary, but Dr. Gordon ran the technology during the field demonstration independently.

The developer states that good organic/analytical laboratory skills and cell culture experience would be useful for successful operation of the technology. Based on observation, the extract cleanup is similar to that used for HRMS sample preparation. Good skills in processing cleanup columns would be important for accurate and precise measurements and how rapidly the samples could be processed. Experience with cell culture would also be useful. Overall, a good technician or entry-level chemist could operate this technology once trained.

Instructions are provided in the form of SOPs once the technology has been licensed. Based on a quick look through the SOPs available at the demonstration, the instructions appeared to be detailed and thorough. Comprehensive training is included with licensing the technology and this would greatly assist the user. This technology has several steps where attention to detail is critical to obtain acceptable sample results. This includes careful processing of samples through the cleanup procedures, pipetting small volumes, and accurately weighing out samples. All standards can be kept at room temperature for a period of one year. After one year, the standards should be remade to ensure confidence. All reagents are valid for one year and should be kept in proper storage (i.e., solvents should be kept in a standard reinforced-metal solvent cabinet at ambient room temperature). Preliminary range finding of the sample extract uses half or less of the extract volume, so there is plenty of extract available to reprocess an analysis without having to re-extract a second sample. This technology can be stopped at several places without adversely affecting sample results, including after extraction, after cleanup, and after solvent evaporation (using a vacuum centrifuge). This technology does require a fair amount of standard laboratory equipment such as an ultrasonic water bath, a vacuum centrifuge, a humidified CO_2 incubator, and a luminometer that could be difficult to troubleshoot in the field if problems occurred. However, a stocked mobile lab would make it convenient to have spare equipment and parts available, and staff would be properly trained in troubleshooting these instruments if any problems were encountered while in the field. This developer will also analyze samples for customers as a fee for service at the developer's location.

7.2.2 Evaluation of Secondary Objective S2: Health and Safety Aspects

Wastes generated with this technology include vials, spent solvent and spent sample from extraction; disposable cleanup columns and solvents from the cleanup steps; and test tubes, solvents, pipette tips, and 96-well plates from the assay. A complete inventory of the waste generated was performed after the demonstration for processing 43 samples by XDS, and the following was recorded. None of the containers was verified as full. Note that this summary does not include the samples that were analyzed in the XDS laboratories.

(1) One 5-gallon container marked “low concentration” containing 58 used acid silica columns, used X-CARB columns, 27 columns, 400 pipette tips, bench paper, and 20 tubes.

(2) One 5-gallon container marked "high concentration" containing 27 caps from soil jars, 23 ampoules from the extract samples, 23 extract tubes, 46 cleanup tubes, 100 pipette tips, and bench paper.

(3) One 5-gallon container with bench paper and 400 culture tubes.

(4) One 5-gallon container with 600 pipette tips, bench paper, and sixteen 96-well plates.

The reader should be advised that, although no difficulties were encountered during this project, difficulties could arise with disposal of dioxin-contaminated waste.

7.2.3 Evaluation of Secondary Objective S3: Portability

As observed, this technology required a fume hood (especially for processing the cleanup columns) and several standard bio-analytical laboratory pieces of equipment such as an ultrasonic water bath, vacuum centrifuge, humidified CO_2 incubator, and a luminometer. Therefore, a trailer with a fume hood would be the minimum required for successful field operation. During the course of the demonstration, the developer also tested a portable airtight chamber that could be used in lieu of a humidified CO_2 incubator. The developer intends for such innovations as the airtight chamber to enhance the field portability of the technology. According to the developer, XDS is working toward increased field portability and is considering equipping its own mobile lab for responding to field requests.

Setup for this demonstration took approximately 8 hours. This included adjusting to a last-minute equipment change by the vendor, who supplied the incubator to Battelle and the developer performing initial maintenance on the instrument essential for its basic operation. The developer believes that having its own mobile lab in the field would greatly reduce setup time, perhaps 1 to 2 hours. With a well-equipped trailer, samples could be processed as efficiently in the field as in the laboratory. For the demonstration, the space constraints of the 28-foot mobile laboratory provided to the developer, including placement of the bench-top double-cabinet incubator on the floor, made processing in the field more cumbersome. In addition, stacked cleanup columns were awkward to process in the short hood height of the mobile lab's fume hood, but the developer made modifications to make the process more manageable.

Differences in reported results due to measurement location (in field vs. laboratory) are described in Section 7.1.6.

7.2.4 Evaluation of Secondary Objective S4: Throughput

XDS processed 43 of the 209 demonstration samples in the field. For the demonstration samples, XDS analyzed the samples once (referred to as "XDS Screen") and reported the results. For greater accuracy, XDS recommends triplicate comprehensive sample analyses so that the average and standard deviation can be reported for the results. These samples were processed by one person and were completed in five days. Approximately 8 hours were lost due to startup meetings and participation in Visitor's Day. Another approximately 8 hours were lost due to a blown hose on the provided CO_2 incubator and an additional malfunction of the instrument. These were failures of the incubator and were out of the developer's control. In view of the condition and failures of the incubator, the developer proceeded more cautiously with dosing the cells, so the final incubations were not completed in batches as large as would have been performed had the incubator not malfunctioned.

The XDS process takes 2 days, with samples being extracted and put through cleanup the first day, incubated overnight, and then results read the second day. The developer felt that one person could process approximately 75 samples during a two-day period and that a two-person team could process even more. Capacity to analyze samples is initially limited by laboratory space and available equipment rather than staffing. A large number of samples can incubate overnight and the read-out of results is relatively quick. The earliest results that would be available from this technology is 36 to 48 hours. The developer states that for samples submitted to XDS for analysis, the standard turnaround time is 30 days; however, preliminary results can be available as quickly as 36 to 48 hours. Based on observation during the demonstration, the target of 75 samples in two days by a single person seems ambitious strictly based on

the time to weigh samples, extract, and clean the extracts; however, some limitations during the demonstration such as the space restrictions (double-cabinet bench-top instrument placed on the floor) and malfunctions of equipment provided to the developer hindered the production process so that optimal production was not observed. In a malfunction-free environment where the operator had conditions set up to ensure optimal production, one person may have been able to process more samples with greater ease. The observer felt that in spite of the limitations that occurred during the demonstration, two people could accomplish 75 samples in two days. The XDS technology is not sold as a kit but rather as a licensed technology or as a fee for service at the XDS laboratory. The technology is based on using 96-well plates. In the range finding portion of the testing, typically 6 to 12 samples can be analyzed depending on what is known about the sample. After this step, 40 samples, a standard curve, and quality control samples can be analyzed per plate, with each plate being read every half-hour.

7.2.5 Miscellaneous Observer Notes

XDS is a U.S. company. Upon licensing the technology, the user is supplied with a complete set of SOPs, full training, and XDS validation of the lab. Samples may also be submitted to XDS for analysis. Phone support is available for both customers who send samples to XDS for analysis and for those licensed to use the technology.

After being licensed, the user would be provided the cells and an initial quantity of the XDS-patented X-CARB used for cleanup columns. Periodically, licensees would need to purchase additional X-CARB from the developer.

Other materials and equipment that the user would need include glass vials with polytetrafluoroethylene-lined caps, 18-mm glass tubes, methanol, toluene, an ultrasonic water bath, a filter, a vacuum centrifuge, hexane, DMSO, the cell culture medium, 96-well culture plates, 2,3,7,8-TCDD standard, a humidified CO_2 incubator, a microscope, a Promega luciferase assay, 5-mL glass disposable pipettes, test tubes, glass columns for the column cleanup, micro-pipettes, a balance, a luminometer, an automated plate shaker, and software for data reduction. In the past, the developer has assisted licensees with acquiring these items.

XDS recommends the following QC with each plate: three blanks, one recovery spike (spiked blank), one matrix spike, one PCDD/F QC standard, one PCB QC standard, four DMSO blanks, and one media blank. Standard sample range-finding analysis is six dilutions of each extract. The dilutions increase accuracy and minimize the need to repeat analyses to generate results within calibration. The number of dilutions varies depending upon what is known of the specific sample (i.e., if the sample is considered low-level, fewer dilutions are needed.) XDS does not recommend a specific frequency of HRMS confirmation of results (they offer European Union regulations as a guide), but it defers this decision to client preference. In general, XDS stated that it would not be as necessary to confirm very high level or very low-level results, but that results near an action level or threshold level for the matrix might benefit from independent confirmation.

Chapter 8
Economic Analysis

During the demonstration, the CALUX® by XDS assay and the reference laboratory analytical methods were each used to perform more than 200 sample analyses, including samples with a variety of distinguishing characteristics such as high levels of PCBs and PAHs. Collectively, the samples provided different levels and types of contamination necessary to properly evaluate the technologies and to perform a comprehensive economic analysis of each technology. The purpose of the economic analysis was to estimate the total cost of generating results by using the CALUX® by XDS assay and then comparing this cost to the reference method. This cost estimate also is provided so that potential users can understand the costs involved with using this technology.

This chapter provides information on the issues and assumptions involved in the economic analysis (Section 8.1), discusses the costs associated with using the CALUX® by XDS assay (Section 8.2), discusses the costs associated with using the reference methods (Section 8.3), and presents a comparison of the economic analysis results for the CALUX® by XDS assay and the reference laboratory (Section 8.4).

8.1 Issues and Assumptions

Several factors affect sample measurement costs. Wherever possible in this chapter, these factors are identified in such a way that decision-makers can independently complete a project-specific economic analysis. The following five cost categories were included in the economic analysis for the demonstration: capital equipment, supplies, support equipment, labor, and investigation-derived waste (IDW) disposal. The issues and assumptions associated with these categories and the costs not included in the analysis are briefly discussed below. The issues and assumptions discussed below only apply to the CALUX® by XDS assay unless otherwise stated.

8.1.1 Capital Equipment Cost

The capital equipment cost was the cost associated with the purchase of the CALUX® by XDS assay. Components of the CALUX® by XDS assay are presented in detail in Chapters 2 and 7. XDS offers a licensing agreement option for potential CALUX® users. Licenses are renewable five-year agreements and include support from XDS in the form of training of client staff, providing laboratory equipment, proprietary software, and laboratory validation. Price information was obtained from a standard price list provided by XDS.

8.1.2 Cost of Supplies

The cost of supplies was estimated based on the supplies required to analyze all demonstration samples using the CALUX® by XDS assay that were not included in the capital equipment cost category. Examples of such supplies include filters, cleanup columns, gas cylinders, solvents, and distilled water. The supplies that XDS used during the demonstration fall into two general categories: consumable (or expendable) and reusable. Examples of expendable supplies utilized by XDS during the demonstration include hexane, toluene, methanol, silica gel, culture flasks, carbon dioxide cylinders, and plastic pipettes. Examples of reusable supplies include a cell culture incubator, low-speed centrifuge, centrifuge concentrator, and a luminometer. It should be noted that this type of equipment may or may not be already owned by a potential CALUX® by XDS assay user; however, for this economic analysis, an assumption was made that the user does not possess these items.

The purchase price of these supplies was either obtained from a standard price list provided by XDS, or it was estimated based on price quotes from independent sources.

XDS is the sole provider of X-CARB (an expendable supply). Recommendations as to where to obtain all other items can be provided by XDS.

8.1.3 Support Equipment Cost

This section details the equipment used at the demonstration such as the mobile laboratory, fume hood, and laptop computer required by the technology. Costs for these items will be reported per actual costs for the demonstration.

8.1.4 Labor Cost

The labor cost was estimated based on the time required for work space setup, sample preparation, sample analysis, and reporting. For the demonstration, developers reported results by submitting a COC/results form. The measurement of the time required for XDS to complete 43 sample analyses in the field (42 labor-hours) was estimated by the sign-in log sheets that recorded the time the XDS operator was on-site. Time was removed for site-specific training activities and Visitor's Day. Additionally, 8 hours was subtracted from the total time XDS spent in the field to account for problems with the CO_2 incubator. Time estimates were rounded to the nearest hour.

During the demonstration, the skill level required for the operators to complete analyses and report results was evaluated. As stated in Section 7.2.1, based on the field observations, a good technician or entry-level chemist could operate this technology once trained, and a single operator could successfully perform the assay. This information was corroborated by XDS.

The education level of the actual field operator was a Ph.D. degree. For the economic analysis, costs were estimated using both actual and projected necessary skill levels for operators.

8.1.5 Investigation-Derived Waste Disposal Cost

During the demonstration, XDS was provided with 5-gallon containers for collecting wastes generated during the demonstration. Sample by-products such as used samples, aqueous and solvent-based effluents generated from analytical processes, used glassware, and PPE were disposed of in the containers. The total cost to dispose of these wastes generated during the demonstration is included in the economic analysis. Items such as coffee cups, food waste, and office waste were disposed of in regular public refuse containers and were not included as IDW and therefore not discussed in this economic analysis.

8.1.6 Costs Not Included

Items whose costs were not included in the economic analysis are identified below along with a rationale for the exclusion of each.

Electricity. During the demonstration, some of the capital equipment was operated using AC power. The costs associated with providing the power supply were not included in the economic analysis as it is difficult to estimate the electricity used solely by the XDS technology. The total cost for electricity usage over the 10-day demonstration was $288. With seven mobile labs/trailers and miscellaneous equipment being operated continuously during the course of the demonstration, the cost of XDS electricity usage would be no more than $41. There was significantly more cost (approximately $13,000) to install an electrical board and additional power at the demonstration site, but this was a function of the demonstration site and not the responsibility of the individual developers, so this cost was not included in the economic analysis.

Oversight of Demonstration Activities. A typical user of the CALUX® by XDS assay would not be required to pay for customer oversight of sample analysis. The EPA, the MDEQ, and Battelle representatives were present during the field demonstration, but costs for oversight were not included in the economic analysis because these activities were project-specific. For these same reasons, cost for auditing activities (i.e., technical systems audits at the reference laboratory and during the field demonstration) were also not included.

Travel and Per Diem for Operators. Operators may be available locally. Because the availability of operators is primarily a function of the location of the project site, travel and per diem costs for operators were not included in the economic analysis.

Sample Collection and Management. Costs for sample collection and management activities, including sample homogenization and labeling, were

not included in the economic analysis because these activities were project-specific and were not dependent upon the selected reference method or developer technology. Additionally, sample shipping, COC activities, preservation of samples, and distribution of samples were specific requirements of this project that applied to all developer technologies and may vary from site to site. None of these costs were included in the economic analysis.

Shipping. Costs for (1) shipping equipment and supplies to the demonstration site and (2) sample coolers to the reference laboratory were not included in the economic analysis because such costs vary depending on the shipping distance and the service used (for example, a courier or overnight shipping versus economy shipping).

Items Costing Less Than $10. The cost of inexpensive items was not included in the economic analysis when the estimated cost was less than $10. Items where it is estimated that the cost was less than $10 included:

- Distilled water
- Personal protective equipment (PPE)
- Waste containers
- Lab stools.

8.2 CALUX® by XDS Costs

This section presents information on the individual costs of capital equipment, supplies, support equipment, labor, and IDW disposal for the CALUX® by XDS assay as well as a summary of these costs. Additionally, Table 8-1 summarizes the CALUX® by XDS costs. As described in Section 4.6, XDS analyzed 43 samples during the field demonstration and 166 samples in its laboratory (total 209 demonstration samples). It is important to note that costs estimated in this section are based on actual costs to analyze the 43 samples during the field demonstration. Cost estimates for analyzing the entire set of 209 demonstration samples were then determined based on the field demonstration costs.

This cost is based on the presumption that the technology would be licensed and used by the user. XDS also offers an analytical service. The cost for XDS to analyze 209 samples is $250 per sample, for a total of $52,250, and does not reflect the usual XDS-provided discounts for this number of samples.

8.2.1 Capital Equipment Cost

The capital equipment cost was the cost associated with the purchase of the technology in order to perform sample preparation and analysis. The CALUX® by XDS assay can be licensed from XDS for $2,400. During the field demonstration, XDS utilized the CALUX® by XDS assay for five days to analyze 43 samples. Because the components of the assay itself are consumable, XDS does not rent the CALUX®; however, the rental of equipment to perform the CALUX® assay is available from XDS.

8.2.2 Cost of Supplies

The supplies that XDS used during the demonstration fall into two general categories: expendable or reusable. Table 8-1 lists all the expendable and reusable supplies that XDS used during the demonstration and the corresponding costs. The cost of each item was rounded to the nearest $1. Expendable supplies are ones that are consumed during the preparation or analysis. Reusable costs are items that must be used during the analysis but ones that can be repeatedly reused. The estimated life of reusable supplies could not be assessed during this economic analysis.

The total cost of the supplies employed by XDS during the demonstration was $40,662. Supplies have to be purchased from a retail vendor of laboratory supplies. Reusable items listed in Table 8-1 can be substituted with other models that operate under the same specifications, thereby modifying the cost of supplies to the potential user.

8.2.3 Support Equipment Cost

XDS analyzed demonstration samples in a 24-foot mobile lab equipped with a fume hood. The rental cost for the mobile lab for use during sample extraction and sample analysis was $2,750. The minimum rental rate for the mobile lab was 1 month. XDS only used the mobile laboratory for five days. Since weekly or daily rental rates for the mobile lab were not an option, the entire cost is reported. As determined by the observers, a construction trailer with a fume hood could have been sufficient for

Table 8-1. Cost Summary

Item	Quantity Used During Field Demo		Unit Cost ($)	Itemized Cost[a] ($) 43 Samples	Itemized Cost[a] ($) 209 Samples
Capital equipment					
Licensing Agreement to use CALUX®	1	unit	2,400	2,400	2,400
Supplies					
Expendable					
5-3/4" Pasteur Pipettes	1	unit	84	84	84
Aluminum Foil	1	unit	5	5	5
Bench-top Paper, 2 rolls of 20" x 300'	1	unit	126	126	126
16 x 125 mm Tubes	1	unit	57	57	57
50-mL glass centrifuge Tubes	1	unit	58	58	58
25-mL Drying Tubes	1	unit	82	82	82
10-mm Drying Tubes	1	unit	72	72	72
Glass Rods	1	unit	130	130	130
Pipet Tips (P200)	1	unit	50	50	50
Pipet Tips (P10)	1	unit	49	49	49
Pipet Tips (P1000)	1	unit	36	36	36
Scintillation Vials	1	unit	110	110	110
Scintillation Vial Caps	1	unit	187	187	187
Silica Gel	1	unit	150	150	150
Sulfuric Acid (2.5-L bottle)	1	unit	54	54	54
Hexane (4-L bottle)	1	unit	22	22	22
Toluene(4-L bottle)	1	unit	36	36	36
Methanol (4-L bottle)	1	unit	44	44	44
Ethyl Acetate (4-L bottle)	1	unit	79	79	79
Acetone (4-L bottle)	1	unit	55	55	55
Celite (500 grams)	1	unit	106	106	106
Sodium Sulfate (1,000 grams)	1	unit	21	21	21
Carbon Matrix (X-CARB)	1	unit	Proprietary	Proprietary	Proprietary
4-mL Teflon Vial	1	unit	33	33	33
13 x 100 Test Tubes	1	unit	29	29	29
DMSO (100 mL)	1	unit	25	25	25
Pipet Bulbs, 2-mL Capacity (pack of 72)	1	unit	39	39	39
Tridecane (25 mL)	1	unit	8	8	8
Glasswool	1	unit	50	50	50
9" Pasteur Pipettes	1	unit	688	688	688
15-mL Plastic Centrifuge Tubes, Sterile	1	unit	163	163	163
50 ml Plastic Centrifuge Tubes	1	unit	207	207	207
Phosphate Buffered Saline (3,000 mL)	1	unit	42	42	42
RPMI Medium (3,000 mL)	1	unit	51	51	51
Trypsin (600 mL)	1	unit	77	77	77
Pen/Strep Solution (600 mL)	1	unit	48	48	48
Fetal Serum (500 mL)	1	unit	104	104	104
Lysis Solution (150 mL)	1	unit	30	30	30
Substrate Solution (10 mL)	1	unit	39	39	39
75 centimeter^2 Tissue Culture Flasks	1	unit	209	209	209
96-Well Plates	1	unit	226	226	226
Backing Tape	1	unit	43	43	43
Ethanol	1	unit	62	62	62
Latex Gloves	1	unit	85	85	85
Pipet Tips, Sterile (P200)	1	unit	30	30	30

Item	Quantity Used During Field Demo		Unit Cost ($)	Itemized Cost[a] ($) 43 Samples	Itemized Cost[a] ($) 209 Samples
2-mL Sterile Pipettes, Plastic (500/case)	1	unit	108	108	108
10-mL Sterile Pipettes, Plastic (200/case)	1	unit	62	62	62
1.0-mL Multipipettor Syringes (100/case)	1	unit	101	101	101
10.0-mL Multipipettor Syringes (100/case)	1	unit	101	101	101
Sodium Hydroxide	1	unit	39	39	39
175 centimer^2 Tissue Culture Flasks	1	unit	176	176	176
75-mL Culture Flasks	1	unit	195	195	195
Cryogenic 2mL Tubes	1	unit	37	37	37
CO_2 Gas Cylinder	1	unit	14	14	14
CO_2 Cylinder Regulator	1	unit	265	265	265
Reusable					
Cell Culture Incubator	1	unit	4,197	4,197	4,197
Centrifuge (Low-Speed, Table Top)	1	unit	915	915	915
Microscope, Inverted	1	unit	400	400	400
Microscope	1	unit	750	750	750
Hemocytometer, Cell Counter	1	unit	105	105	105
Shaker for 96-Well Plates	1	unit	790	790	790
Balance	1	unit	2,500	2,500	2,500
Centrifuge Concentrator	1	unit	3,500	3,500	3,500
Sonicating Water Bath	1	unit	506	506	506
Luminometer	1	unit	22,000	22,000	22,000
Support Equipment					
Mobile Laboratory	1	unit	2,750	2,750	2,750
Laptop Computer	1	unit	1,000	1,000	1,000
Labor					
Operator	42	labor hours	80[b]	3,360	16,331
IDW Disposal[c]	1	unit	292	292	1,419
Total Cost if performed all 209 in field				**$48,064**	**$62,162**
Total Cost as performed (43 samples in field and 166 in XDS laboratory)				**$48,064**	**$89,564**
Total Cost if Performed by XDS in its laboratory				**$10,750**	**$52,250**

[a] Itemized costs were rounded to the nearest $1.

[b] Labor rate for field technicians to operate technology rather than research scientists was $50.75 an hour, $2,132 for 43 samples and $10,360 for 209 samples.

[c] Further discussion about waste generated during demonstration can be found in Chapter 7.

operation of this technology in the field. Use of a construction trailer with a fume hood would have been more cost efficient, lowering the support equipment cost by at least $1,000.

A laptop computer is a necessary for the efficient operation of this technology. This is a one-time purchase that is reusable.

8.2.4 Labor Cost

As described in Section 8.1.4, 42 labor-hours were spent in the field to analyze 43 samples. An hourly rate of $32.10 was used for a research scientist performing sample extractions and sample analysis, and a multiplication factor of 2.5 was applied to labor costs in order to account for overhead costs.[9] Based on this hourly rate and multiplication factor, a labor rate of $3,360 was determined for the analysis of the 43 samples during the field demonstration. It was estimated that the labor cost for the total 209 samples was $16,331.

Based on observation, it is anticipated that lower-cost field technicians, with proper training and skill levels, could have analyzed the samples in a similar amount of time. As such, the labor rate for the analysis of 43 samples during the field demonstration could have been as low as $2,132 (hourly rate of $20.30 with 2.5 multiplication factor for 42 labor-hours), and $10,360 for all 209 demonstration samples.

8.2.5 Investigation-Derived Waste Disposal Cost

As discussed in Chapter 7, XDS was provided with 5-gallon containers for collecting wastes generated during the demonstration. Chapter 7 discusses the type and amount of waste generated by the technology during the field demonstration in more detail.

During the demonstration, XDS analyzed 43 samples. The total cost to dispose of the waste generated for these samples was $292. The cost to dispose of waste for all 209 samples is estimated at $1,419.

8.2.6 Summary of CALUX® by XDS Costs

The total cost for performing dioxin and PCB analyses using the CALUX® by XDS assay in the field for all 209 samples was $62,162. The dioxin and PCB analyses were performed for 58 soil and sediment PE samples, 128 soil and sediment environmental samples, and 23 extracts. When XDS performed multiple dilutions for a sample, these were not included in the number of samples analyzed. The cost to have XDS analyze the 209 samples as an analytical service would have been $52,250. The cost to analyze the samples as it was performed (43 samples in the field and 166 samples in the XDS laboratories) was $89,564.

The total cost of $62,162 for analyzing the demonstration samples under the CALUX® by XDS licensing option included $2,400 for capital equipment (licensing agreement); $40,662 for supplies; $3,750 for support equipment; $16,331 for labor; and $1,419 for IDW disposal. Of these five costs, the largest cost was for the supplies (65% of the total cost).

8.3 Reference Method Costs

This section presents the costs associated with the reference method used to analyze the 209 demonstration samples for dioxin and dioxin-like PCBs. Typical costs of these analyses can range from $800 to $1,100 per sample, depending on the method selected, the level of quality assurance/quality control incorporated into the analyses, and reporting requirements. The reference laboratory utilized EPA Method 1613B for D/F analysis and EPA Method 1668A for coplanar PCB analysis for all soil and sediment samples for comparison with the CALUX®. The reference method costs were calculated using cost information from the reference laboratory invoices.

Table 8-2 summarizes the projected and actual reference method costs. At the start of the demonstration, the reference laboratory's projected cost per sample was $785 for D/F analysis and $885 for PCB analysis. This cost covered the preparation and analysis of the demonstration samples, required method QC samples, electronic data deliverable, and the data package for each. The actual cost for the 209 demonstration analyses was $213,580 for D/F and $184,449 for PCBs, and a total of $398,029. This was higher than the projected ($321,380) due to reanalysis, re-extractions, dilutions and additional cleanups that were above the 30% repeats allowable by the original quote. The turnaround time by the reference laboratory for reporting all 209 samples was approximately eight

months (171 business days). The quoted turnaround time was three months.

8.4 Comparison of Economic Analysis Results

The total costs for the CALUX® by XDS ($89,564) and the reference method ($398,029) are listed in Tables 8-1 and 8-2, respectively. The total cost for the CALUX® by XDS was $308,465 less than the reference method. It should be noted that XDS analyzed 43 samples in five days on-site during the demonstration and completed the remaining 166 samples in its laboratory within six weeks of the demonstration. XDS reports a typical (non-expedited) turnaround time of 21 to 30 days for sample analyses in their laboratory. The demonstration analyses took slightly longer than normal due to the volume of samples and other sample analyses already in their queue. For comparison, the reference laboratory took 8 months to report all 209 samples.

Use of the CALUX® by XDS assay in the field will likely produce additional cost savings because the results will be available within a few hours of sample collection; therefore, critical decisions regarding sampling and analysis can be made in the field, resulting in a more complete data set. Additional possible advantages to using field technologies include reduction of multiple crew and equipment mobilization-demobilization cycles to a single cycle, dramatically increased spatial resolution mapping for higher statistical confidence, leading to reduced insurance costs and reduced disposal costs, and compression of total project time to reduce administrative overhead. However, these savings cannot be accurately estimated and thus were not included in the economic analysis. Project-specific costs associated with the use of the technology, such as the cost for HRMS confirmation analyses and training costs to be proficient in the use of the technology, were also not accounted for in this analysis.

CALUX® by XDS is a method that reports both $TEQ_{D/F}$ and TEQ_{PCB}. The reference method reports these TEQ values as well as concentrations for individual congeners. Although the CALUX® by XDS analytical results did not have the same level of detail as the reference method analytical results (or comparable QA/QC data), the CALUX® by XDS assay provided D/F and coplanar PCB analytical results on-site at significant cost and time savings compared to the reference laboratory.

Table 8-2. Reference Method Cost Summary

Analyses Performed	Number of Samples Analyzed	Cost per sample Quotation ($)	Itemized Cost ($)	
			Quotation[a]	Actual
Dioxin/Furans, EPA Method 1613B, GC/HRMS	23 extracts	735	16,905	213,580
	186 soil/sediment	785	146,010	
WHO PCBs EPA Method 1668A, GC/HRMS	23 extracts	685	15,755	184,449
	186 soil/sediment	735	136,710	
1668 Optional Carbon Column DB1	40	150	6,000	
Total Cost	209 samples		**321,380**	**398,029**

[a] Price includes up to 30% of samples requiring additional work of some kind (dilutions or extra cleanup). Greater than that would require additional work with further charges associated to them ($150 to $180 per sample per procedure).

Chapter 9
Technology Performance Summary

The purpose of this chapter is to provide a performance summary of the CALUX® by XDS by summarizing the evaluation of the primary and secondary objectives of this demonstration in Tables 9-1 and 9-2, respectively. Detailed information about these evaluations, including a complete evaluation of the reference laboratory data, can be found in previous sections of this report.

When comparing the CALUX® by XDS results with HRMS TEQ results from the certified samples and the reference methods, the reader should keep in mind the limitations of the TEQ approach described in Section 4.2. Note that it is possible that Ah-receptor binding compounds that are being measured during the CALUX® by XDS analysis are not all accounted for in the reference laboratory TEQ result and that the 1998 WHO TEFs used to generate the reference laboratory TEQs may differ from the assay Ah-receptor binding affinity for certain analytes. The data generated and evaluated during this demonstration showed that the XDS technology was not directly comparable to the HRMS TEQ values in many cases. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint. However, it showed it could be an effective tool to screen for samples above or below 1 pg/g TEQ for $TEQ_{D/F}$ and total TEQ and above or below 50 pg/g TEQ for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ, particularly considering that both the cost ($89,564 vs. $398,029) and the time (six weeks vs. eight months) to analyze the 209 demonstration samples were significantly less than that of the reference laboratory.

Table 9-1. CALUX® by XDS System Performance Summary - Primary Objectives

Objective	Performance			
	Statistic	TEQ_{PCB}	$TEQ_{D/F}$	Total TEQ
P1: Accuracy	Number of data points	6	8	10
	Median Recovery (%)	25	307	141
	Mean Recovery (%)	548	514	217
P2: Precision	Number of data points	22	47	49
	Median RSD (%)	97	32	42
	Mean RSD (%)	105	41	53
P3: Comparability	Number of data points	105	180	168
	Median RPD (%)	-17	-102	-92
	Interval agreement (%)	82	69	72
	Blank agreement (%)	25	75	not determined
P4: Estimated Method Detection Limit	EMDL (pg/g)	not determined	0.53 to 0.63	not determined
P5: False Positive/False Negative Rate	False positive rate at 1 pg/g TEQ (%)	15%	6%	4%
	False positive rate at 50 pg/g TEQ (%)	9%	10%	6%
	False negative rate at 1 pg/g TEQ (%)	23%	0%	1%
	False negative rate at 50 pg/g TEQ (%)	6%	0.5%	0%
P6: Matrix Effects	• Measurement location: 21% statistically different • Matrix type: none • Sample type: slight for total TEQ • PAH concentration: none • Environmental site: none • Known interferences: slight			
P7: Cost	As demonstrated, total cost was $89,564 (43 samples during field demonstration: $48,064 and 166 samples analyzed by XDS in its laboratories: $41,500). Projected if all 209 demonstration samples were analyzed in field: $62,162. Projected if all 209 demonstration samples were analyzed in XDS laboratory: $52,250.			

Table 9-2. CALUX® by XDS System Performance Summary - Secondary Objectives

Objective	Performance
S1: Skill level of Operator	Good skills in processing cleanup columns would be important for accurate and precise measurements and how rapidly the samples could be processed. Experience with cell culture would also be useful. Overall, a good technician or entry-level chemist could operate this technology once trained.
S2: Health and Safety Aspects	Wastes generated with this technology include vials, spent solvent, and spent sample from extraction; disposable cleanup columns and solvents from the cleanup steps; and test tubes, solvents, pipette tips, and 96-well plates from the assay. Cost for waste disposal of 209 samples was estimated at $1,419. A fume hood is necessary for the operation of this technology.
S3: Portability	In addition to a fume hood, this technology required several standard bio-analytical laboratory pieces of equipment such as an ultrasonic water bath, vacuum centrifuge, humidified CO_2 incubator, and a luminometer. Therefore, a trailer with a fume hood would be the minimum required for successful field operation. XDS is working toward increased field portability and is considering equipping its own mobile lab for responding to field requests.
S4: Sample Throughput	During the field demonstration, 43 samples were processed by XDS, equating to a sample throughput rate of 9 samples per day. This was accomplished in about five full working days (42 labor-hours), with one person exclusively performing the work. (See Section 7.2.4 regarding nondeveloper-related instrumentation problems and throughput delays.) XDS reported the remaining sample 166 results that were analyzed in their laboratories in six weeks (normal, nonexpedited turnaround times are 21 to 30 days).

Chapter 10
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Appendix A
SITE Monitoring and Measurement Technology Program
Verification Statement

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Office of Research and Development
Washington, DC 20460

SITE Monitoring and Measurement Technology Program Verification Statement

TECHNOLOGY TYPE:	**Aryl Hydrocarbon Receptor Bioassay**
APPLICATION:	**MEASUREMENT OF DIOXIN AND DIOXIN-LIKE COMPOUNDS**
TECHNOLOGY NAME:	**CALUX® by XDS**
COMPANY:	**Xenobiotic Detection Systems, Inc.**
ADDRESS:	**1601 E. Geer Street, Suite S** **Durham, North Carolina 27704**
PHONE:	**(919) 688-4804**
WEB SITE:	**www.dioxins.com**
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VERIFICATION PROGRAM DESCRIPTION

The U.S. Environmental Protection Agency (EPA) created the Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program to facilitate deployment of innovative technologies through performance verification and information dissemination. The goal of this program is to further environmental protection by substantially accelerating the acceptance and use of improved and cost-effective technologies. The program assists and informs those involved in designing, distributing, permitting, and purchasing environmental technologies. This document summarizes results of a demonstration of Chemical-Activated LUciferase eXpression (CALUX®) by Xenobiotic Detection Systems, (XDS) Inc.

PROGRAM OPERATION

Under the SITE MMT Program, with the full participation of the technology developers, the EPA evaluates and documents the performance of innovative technologies by developing demonstration plans, conducting field tests, collecting and analyzing demonstration data, and preparing reports. The technologies are evaluated under rigorous quality assurance protocols to produce well-documented data of known quality. The EPA's National Exposure Research Laboratory, which demonstrates field sampling, monitoring, and measurement technologies, selected Battelle as the verification organization to assist in field testing technologies for measuring dioxin and dioxin-like compounds in soil and sediment.

DEMONSTRATION DESCRIPTION

The demonstration of technologies for the measurement of dioxin and dioxin-like compounds was conducted at the Green Point Environmental Learning Center in Saginaw, Michigan, from April 26 to May 5, 2004. The primary objectives for the demonstration were as follows:

P1. Determine the accuracy.
P2. Determine the precision.
P3. Determine the comparability of the technology to EPA standard methods.
P4. Determine the estimated method detection limit (EMDL).
P5. Determine the frequency of false positive and false negative results.
P6. Evaluate the impact of matrix effects on technology performance.
P7. Estimate costs associated with the operation of the technology.

The secondary objectives for the demonstration were as follows:

S1. Assess the skills and training required to properly operate the technology.
S2. Document health and safety aspects associated with the technology.
S3. Evaluate the portability of the technology.
S4. Determine the sample throughput.

A total of 209 samples was analyzed by each technology, including a mix of performance evaluation (PE) samples, environmentally contaminated samples, and extracts. XDS analyzed 43 of these samples during the field demonstration and 166 samples in their laboratory. The PE samples were used primarily to determine the accuracy of the technology and consisted of purchased reference materials with certified concentrations. The PE samples also were used to evaluate precision, comparability, EMDL, false positive/negative results, and matrix effects. Dioxin-contaminated samples from Warren County, North Carolina; the Saginaw River, Michigan; Tittabawassee River, Michigan; Midland, Michigan; Winona Post, Missouri; Nitro, West Virginia; Newark Bay, New Jersey; Raritan Bay, New Jersey; and Brunswick, Georgia were used to evaluate precision, comparability, false positive/negative results, and matrix effects. Extracts prepared in toluene were used to evaluate precision, EMDL, and matrix effects. All samples were used to evaluate qualitative performance objectives such as technology cost, the required skill level of the operator, health and safety aspects, portability, and sample throughput. AXYS Analytical Services (Sidney, British Columbia) was contracted to perform the reference analyses by high-resolution mass spectrometry (HRMS) (EPA Method 1613B and EPA Method 1668A) to compare to the CALUX® by XDS assay. The purpose of the verification statement is to provide a summary of the demonstration and its results; detailed information is available in *Technologies for Monitoring and Measurement of Dioxin and Dioxin-like Compounds in Soil and Sediment—Xenobiotic Detection Systems CALUX® by XDS* (EPA/540/R-05/001).

TECHNOLOGY DESCRIPTION

The technology description and operating procedure below are based on information provided by XDS. XDS has patented (U.S. patent number 5,854,010) a genetically engineered cell line that contains the firefly luciferase gene under transactivational control of the aryl hydrocarbon (Ah) receptor. This cell line can be used for the detection and quantification of the Ah receptor agonists, the target receptor of dioxins, furans, and polychlorinated biphenyls (PCBs). The XDS term for the in vitro assay is the CALUX® by XDS assay. The most widely studied compounds that activate this system are the polychlorinated diaromatic hydrocarbons (PCDH), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Many PCDH compounds are quantified relative to TCDD, since this is one of the most potent activators of Ah-receptor mediated gene transcription. These relative quantifications are known as toxicity equivalents (TEQs), and the results from the CALUX® by XDS assay provide a measure of TEQs in a sample. By using patented cleanup methods developed by XDS (U.S patent number 6,720,432 B2), it is possible to separate PCBs from dioxins/dibenzofurans and to determine what portion

of the total TEQ in a sample is due to each of these classes of compounds. XDS has termed this procedure the Dioxin/Furan and PCB-Specific (DIPS) or DIPS-CALUX® by XDS bioassay. The TEQs were reported individually for dioxins/furans and PCBs.

VERIFICATION OF PERFORMANCE

The CALUX® by XDS technology is an Ah-receptor bioassay that individually reports total dioxin/furan TEQ ($TEQ_{D/F}$) and total PCB TEQ (TEQ_{PCB}) in picogram/gram (pg/g). When comparing the CALUX® by XDS results with HRMS TEQ results from the certified samples and the reference methods, the reader should keep in mind the limitations of the TEQ approach, noting that it is possible that Ah-receptor binding compounds that are being measured during the CALUX® by XDS analysis are not all accounted for in the reference laboratory TEQ result and that the World Health Organization toxicity equivalency factors used to generate the reference laboratory TEQs may differ from the assay Ah-receptor binding affinity for certain analytes. Therefore, the technology should not be viewed as producing an equivalent measurement value to HRMS TEQ values for all samples. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint.

Accuracy: The determination of accuracy was based on the agreement of the XDS results with the certified or spiked levels of the PE samples that were obtained from commercial sources. Accuracy was assessed by percent recovery (R), which is the average of the replicate results from CALUX® by XDS divided by the certified or spiked value of the PE sample, multiplied by 100%. Ideal R values are near 100%. The overall R values were 548% (mean), 25% (median), 3% (minimum), and 1,736% (maximum) for TEQ_{PCB} values; 514% (mean), 307% (median), 120% (minimum), and 1,842% (maximum) for $TEQ_{D/F}$ values; and 217% (mean), 141% (median), 15% (minimum), and 868% (maximum) for total TEQ values.

Precision: Replicates were incorporated for all samples (PE, environmental, and extracts) included in the 209 samples analyzed in the demonstration. Three samples had seven replicates in the experimental design, one sample had eight replicates, and all other samples had four replicates. Precision was determined by calculating the standard deviation of the replicates, dividing by the average concentration of the replicates, and multiplying by 100%. Ideal RSD values are less than 20%. The overall RSD values were 105% (mean), 97% (median), 26% (minimum), and 199% (maximum) for TEQ_{PCB}; 41% (mean), 32% (median), 2% (minimum), and 124% (maximum) for $TEQ_{D/F}$; and 53% (mean), 42% (median) , 3% (minimum), and 165% (maximum) for total TEQ.

Comparability: The XDS results were compared to EPA Method 1613B and 1668A results. The results were compared by determining the relative percent difference (RPD) by dividing the difference of the two numbers by the average of the two numbers and multiplying by 100%. Ideal RPD values are between positive and negative 25%. The overall RPD values were -17% (median), -200% (minimum), and 200% (maximum) for TEQ_{PCB}; -102% (median), -198% (minimum), and 196% (maximum) for $TEQ_{D/F}$; and -92% (median), -191% (minimum), and 186% (maximum) for total TEQ. The XDS results were also compared to the reference laboratory results using an interval approach to determine if the XDS results and the reference laboratory results would place the samples in the same action-level interval, thereby resulting in the same action-oriented decision. The developer and reference data were grouped into four TEQ concentration ranges. The ranges were $\leq$ 50 pg/g, 50 to 500 pg/g, 500 to 5,000 pg/g, and $\geq$ 5,000 pg/g. The intervals were determined based on current guidance for cleanup levels. The percentage of developer results that agreed with reference laboratory results was 82% for TEQ_{PCB}, 69% for $TEQ_{D/F,}$ and 72% for total TEQ.

Estimated method detection limit: EMDL was calculated generally according to the procedure described in 40 CFR Part 136, Appendix B, Revision 1.11. Lower EMDL values indicate better sensitivity. The calculated EMDLs ranged from 0.53 to 0.63 pg/g $TEQ_{D/F}$, depending on whether nondetect values were assigned values of zero, one-half the reporting limit value, or the reporting limit value itself. The detection limit reported by XDS in the demonstration plan was 0.3 pg/g $TEQ_{D/F}$.

False positive/negative results: Samples that were reported as less than a specified level by the reference laboratory but greater than the specified level by XDS were considered false positive. Conversely, those samples that were reported as less than the specified level by XDS but reported as greater than the specified level by the reference laboratory were considered false negatives. Ideal false positive and false negative rates were zero. The technology had a fairly high rate of false positive and false negative results around 1 pg/g TEQ_{PCB} (15% and 23%, respectively), but it had significantly fewer false positives and false negatives for total TEQ (4% and 1%, respectively) and $TEQ_{D/F}$ (6% and 0%, respectively). When comparing XDS's results to the reference laboratory for samples above and below 50 pg/g TEQ, all of the false positive and false negative rates for all TEQ types were less than 10%. These data suggest that the XDS technology could be an effective tool to screen samples above or below 1 pg/g TEQ for $TEQ_{D/F}$ and total TEQ, and that it could be effective for all three types of TEQ values to determine results above or below 50 pg/g TEQ.

Matrix effects: The likelihood of matrix-dependent effects on performance was investigated by evaluating results in a variety of ways. The XDS results that were generated in the laboratory and in the field for replicate samples were statistically different for 21% of the samples, and, in these cases, the XDS laboratory result was generally more comparable to the reference laboratory. No significant effect was observed for the reproducibility of XDS results by matrix type (soil, sediment, and extract) or by PAH concentration. A slight effect was observed for total TEQ when comparing XDS's results by sample type (PE vs. environmental vs. extract), but $TEQ_{D/F}$ and TEQ_{PCB} showed no statistical difference. PE samples spiked for a particular contaminant (e.g., PCBs) were sometimes reported as detections for other analytes that were not spiked in the sample (e.g., D/Fs). The XDS results were not more or less comparable to the reference laboratory results based on environmental site.

Cost: The cost of the technology was documented and compared to the cost of the reference analyses. As demonstrated, the total cost for the CALUX® by XDS to analyze all 209 samples was $89,564. The cost for the reference laboratory to analyze all 209 samples by Method 1613B and Method 1668A was $398,029. The total cost for the CALUX® by XDS was $308,465 less than the reference method.

Skills and training required: Based on observation during the field demonstration, good skills in processing cleanup columns would be important for accurate and precise measurements and how rapidly the samples could be processed. Experience with cell culture would also be useful. Overall, a good technician or entry-level chemist could operate this technology once trained.

Health and safety aspects: Wastes generated with this technology include vials, spent solvent, and spent sample from extraction; disposable cleanup columns and solvents from the cleanup steps; and test tubes, solvents, pipette tips, and 96-well plates from the assay. A fume hood is necessary for the operation of this technology.

Portability: In addition to a fume hood, this technology required several standard bioanalytical laboratory pieces of equipment such as an ultrasonic water bath, vacuum centrifuge, humidified CO_2 incubator, and luminometer. Therefore, a trailer with a fume hood would be the minimum required for successful field operation.

Sample throughput: During the field demonstration, 43 samples were processed by XDS, equating to a sample throughput rate of 9 samples per day. This was accomplished in about 5 full working days (42 labor-hours), with one person exclusively performing the work. (See Section 7.2.4 regarding nondeveloper-related instrumentation problems and throughput delays.) XDS completed the remaining 166 samples in their laboratory within 6 weeks of the demonstration. (Note that typical, non-expedited turnaround times are 21 to 30 days in the XDS laboratory.) For comparison, the reference laboratory took 8 months to report all 209 samples.

Appendix B
Supplemental Information Supplied by the Developer

The purpose of this section is for the developer to provide additional information about the technology. This can include updates/changes/modifications planned for the technology or which have occurred since the technology was tested. The developers can also use this section to comment and expand on the findings of the report.

Xenobiotic Detection Systems Comments on EPA Site Program Data

Xenobiotic Detection Systems is pleased to have been invited and to have participated in this study. The EPA and Battelle have run a rigorous cross validation study comparing our XDS CALUX screening estimates of dioxin-like chemicals to high resolution gas chromatography/mass spectrometry analysis of chlorinated dioxins/furans and PCBs. This was a highly complex project demanding analytical precision over 7 logs of concentration for these toxic chemicals, and was an exceedingly difficult accomplishment for any analytical procedure.

XDS is proud of the characterization of the XDS CALUX technology provided in this report. It clearly illustrates the value of our technology in examining toxicity issues in soil and sediment samples. The study also demonstrates the technology is applicable to other matrices and situations.

> "These data suggest that the XDS technology could be an effective tool to screen samples as being above or below 1 pg/g TEQ or TEQ D/F and total TEQ, and that it could be effective for all three types of TEQ values to determine results above or below 50 pg/g."
>
> "The total cost for the CALUX by XDS to analyze all 209 samples was $89,564. The cost for the reference laboratory to analyze all 209 samples by Method 1613B and Method 1668A was $398,029. The total cost for the CALUX by XDS was $308,465 less than the reference method.
>
> "During the field demonstration, 43 samples were processed by XDS….in about 5 full working days. XDS completed the remaining 166 samples in their laboratory within 6 weeks of the demonstration. For comparison, the reference laboratory took 8 months to report all 209 samples."

XDS CALUX was designed to be a screening tool to evaluate contamination by these chemicals. The bioassay was able to provide estimates of contamination particularly at the action levels of regulatory agencies for soil samples and at significant cost savings. The method detection limit was determined to be between 0.53 pg to 0.63 pg TEQ/g sample and provides sufficient sensitivity for screening samples at 1 pg/g TEQ and 50 pg/g TEQ for dioxins/furans yielding approximately 6 % false positives and 0% false negatives.

The screening mode of the XDS CALUX analysis used in this study entails extraction of the sample once, processing with a single determination at a variety of dilutions of the extract to provide a crude estimate of the concentration of dioxin-like activity. This screening mode for the XDS-CALUX assay is not appropriate for defining precision and accuracy with any confidence. However, our quantitative mode of analysis is more appropriate to provide this level of sample detail in an environment of high confidence and at detection levels below one part per trillion.

Due to fiscal, time constraints, and the need to demonstrate the technology in the field in this study, our participation was limited to providing the screening analyses of these EPA-Battelle samples. A more appropriate XDS CALUX analysis for defining precision and accuracy is provided by our quantitative mode of the assay using triplicate analysis (preparing three individual extracts from a single sample and analyzing these independently). This comprehensive triplicate analysis allows for the determination of the relative standard deviation (RSD) of the analyses and this figure is generally less than 20%.

Information was provided by the developer and does not necessarily reflect the opinion of the EPA.

Below is a chart (Figure 1) demonstrating the relationship between results on chlorinated dioxins/furan TEQ provided by XDS CALUX analyses for the EPA-Battelle samples verses the reference GC/MS laboratory results using a log-log plot.

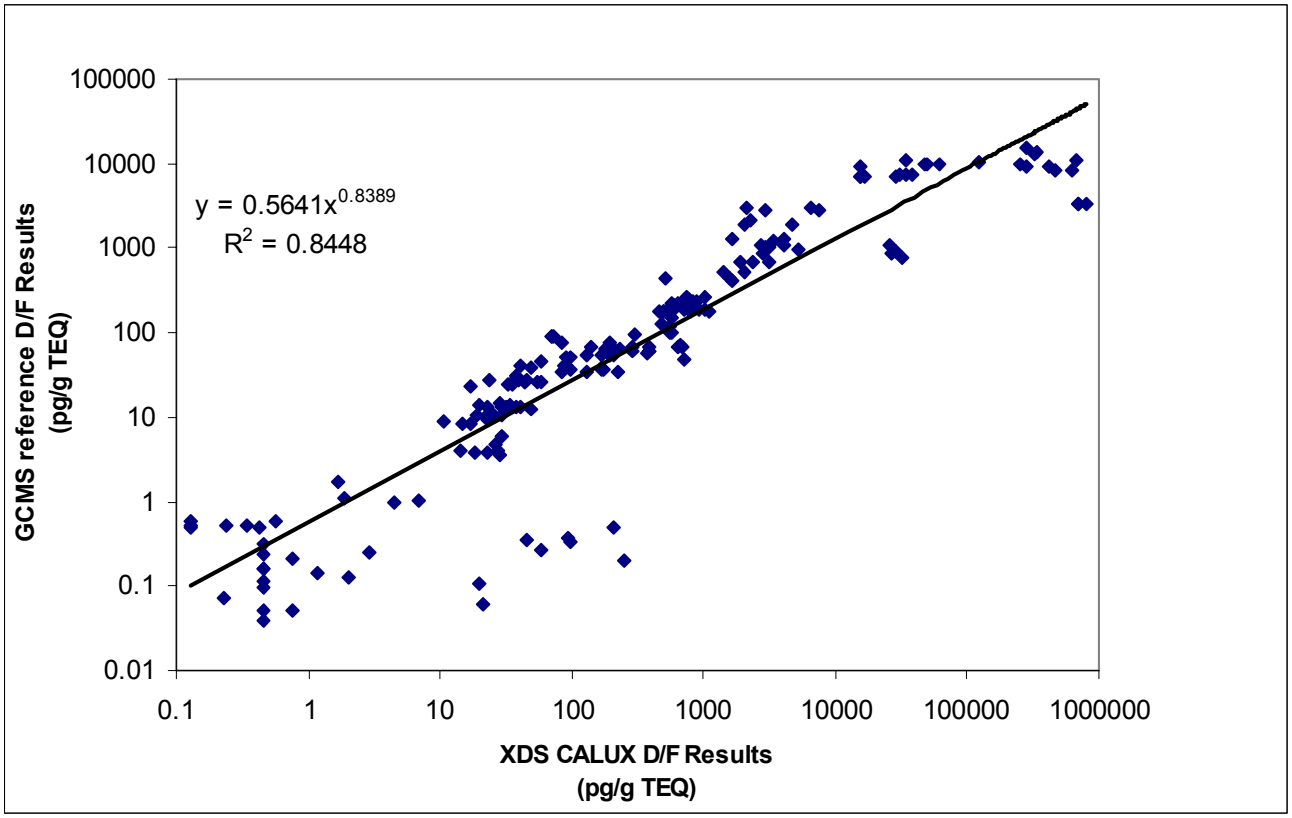

Figure 1

The data correlate well ($R^2 = 0.8448$).

These screening data points do not demonstrate a strict one to one correspondence. This is expected since the TEQ estimates by XDS CALUX receptor based technology provides an estimate of activation of the receptor by the chemical extract. Many biological responses are logarithmically related to concentration. The plotting of this relationship generates one model that describes the relationship.

The deviation from a direct relationship occurs for a number of reasons including such factors as presence of other halogenated dioxins and furans, differences in the REP values of XDS cells versus the TEF values used to scale the GC/MS estimates of TEQ, and the kinetics of binding and activation of the receptor. Modeling the data we can derive a formula to transform the CALUX data to provide a better estimate of the GC/MS data.

The formula for Figure 2 is $y = 0.8389x - 0.2467$, where y = log GC/MS and x = log CALUX.

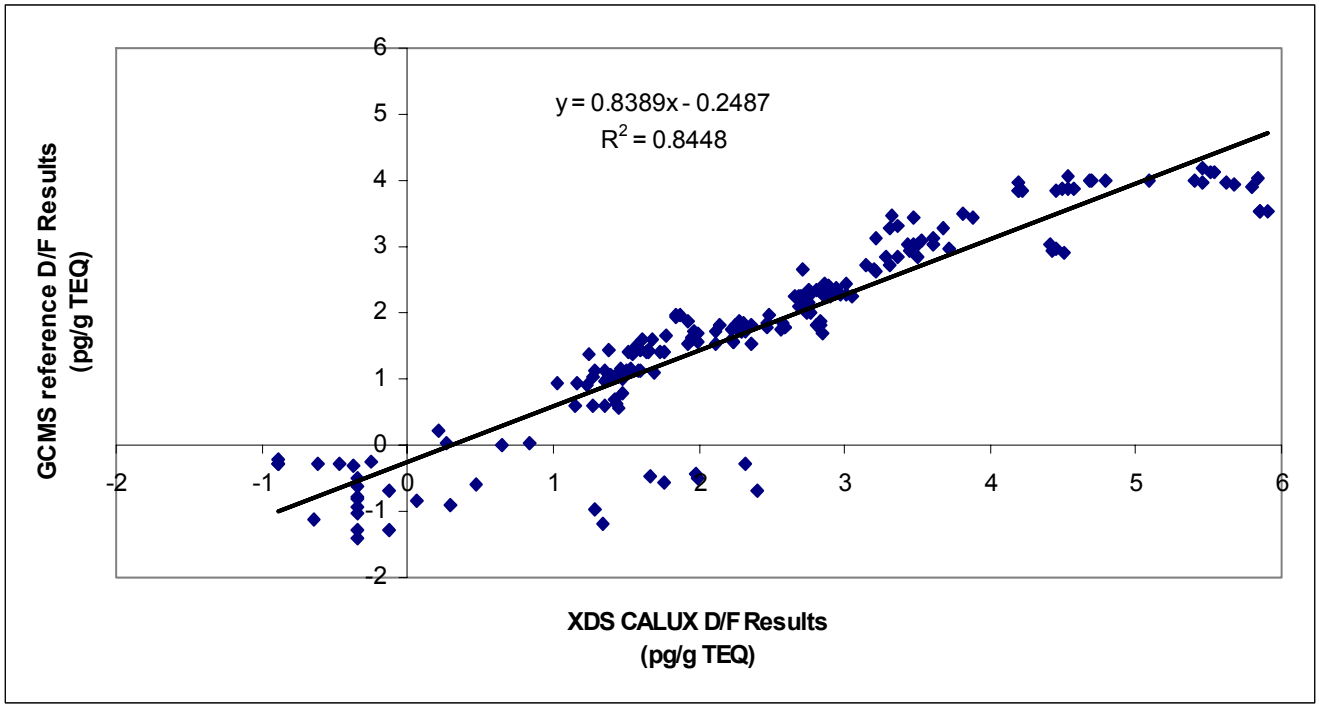

Figure 2

Determining the appropriate model would improve the comparability of the GC/MS and XDS-CALUX estimates of dioxin/furan and Total TEQ concentrations. The XDS CALUX method does underestimate the concentration of PCBs. This is due to the differences in the relative response factors for these chemicals and the WHO TEF values used to scale the GC/MS data.

A point to be noted in the execution of this study is that many of the developers requested or required information about the contaminant levels of the provided samples. Xenobiotic Detection Systems chose not to accept any prior information on any of the sample materials and preferred to keep the study conditions completely double blind and much closer to a real-world analysis scenario.

Savings

This report cited the large difference in the cost of the XDS CALUX analyses and the reference laboratory analyses. Our clients are already aware of this and many use the XDS technology to screen their samples and reduce their need for the more expensive GC/MS congener-specific analyses. The table (Figure 3) below illustrates the savings of using XDS CALUX in conjunction with a GC/MS follow-up confirmation analysis. This table uses the XDS CALUX costs ($89,564) for the analyses of the 209 SITE samples and the actual cost of the SITE reference laboratory analyses ($398,029).

209 Samples	CALUX by XDS Screening Analysis	GC/MS Analysis	Screening plus GC/MS	Savings vs. 100% GC/MS
No follow-up required	$89,564	$0	$89,564	$308,372
1% GC/MS Follow-up	$89,564	$5,712	$95,276	$302,660
2% GC/MS Follow-up	$89,564	$9,520	$99,084	$298,852
5% GC/MS Follow-up	$89,564	$20,944	$110,508	$287,428
10 % GC/MS Follow-up	$89,564	$39,984	$129,548	$268,388
20 % GC/MS Follow-up	$89,564	$79,968	$169,532	$228,404
30 % GC/MS Follow-up	$89,564	$119,952	$209,516	$188,420
40 % GC/MS Follow-up	$89,564	$159,936	$249,500	$148,436
50 % GC/MS Follow-up	$89,564	$199,920	$289,484	$108,452
60 % GC/MS Follow-up	$89,565	$239,904	$329,469	$68,467
70 % GC/MS Follow-up	$89,566	$279,888	$369,454	$28,482
100% GC/MS	No CALUX Analyses	$398,029		

Figure 3

XDS Clients

Xenobiotic Detection Systems holds and regards the names of clients as highly confidential information. We do not release client names or provide any client information, without the client's consent. We observe this same confidentiality policy in regard to our CALUX by XDS licensees.
XDS has clients in the animal feed industry, environmental consulting and engineering companies, food producers, leading colleges and universities, municipalities, incineration plants, manufacturing industries and other categories. Additional information, including research abstracts, is available on the XDS web site, www.dioxins.com.

If you are interested in contacting current XDS CALUX clients or licensees, please contact Xenobiotic Detection Systems.

We welcome opportunities to further explain and answer questions on our CALUX by XDS technology. Please contact us at info@dioxins.com or 1-888-DIOXINS (346-9467).

Summary:

Advantages of using CALUX by XDS:

209 SITE samples	CALUX by XDS	Reference Laboratory
Cost	$ 89,564	$398,029
Time	6 weeks*	8 months

*If necessary, the SITE samples could have been analyzed within a three- to four-week period. Our normal (non-expedited) turn around time for analyses is 21 to 30 days.

Faster than GC/MS

Results available in hours and days verses weeks

Less expensive than GC/MS

Costs are in hundreds of dollars verses one thousand dollars or more

Flexible - as screening detection levels and threshold action levels can be client specific

Sensitive - detecting Dioxin/Furans below 1 ppt

Accurate - results are reproducible

Multiple samples can be processed during the same analysis procedure.

Already accepted in the European Union as a screening tool for foodstuffs

Can be rapidly set up in remote mobile facilities with minimal construction

Requires standard laboratory equipment, not excessive expensive instrumentation

Minimal laboratory staff required

Appendix C
Reference Laboratory Method Blank and Duplicate Results Summary

Table C-1. Summary of Method Blank Performance

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
D/F WG12107	Y	0.000812	26.1–74.1 (Newark Bay) 9.93–13.3 (Raritan Bay)	
D/F WG12148	N	0.133	13.5–50.4 (Newark Bay) 49.5–15,200 (Brunswick)	Many samples had concentrations >20x blank. Few that didn't were not significantly affected on a total TEQ basis.
D/F WG12264	N	0.0437	1.0–94.1 (Titta. River sediment) 0.237–6,900 (PE)	Most samples had concentrations >20x blank. Low level Tittabawassee River sediment samples L6749-2 (Ref 48[b]), -9 (Ref 130), -10 (Ref 183), and -12 (Ref 207) were evaluated based on their replication within the demonstration analyses and comparison to characterization results and considered unaffected by method blank exceedances. Low level PE samples L6760-1 (Ref 25), –3 (Ref 28), and -4 (Ref 29) were D/F blanks with resulting TEQs sufficiently low enough to still be distinguished as blank samples.
D/F WG12534	N	0.610	25.3–7,100 (PE)	Sample concentrations > 20x blank.
D/F WG12641	N	0.0475	31–269 (Midland) 72.8 (Brunswick) 123 (Titta. River sediment) 0.159–7,690 (PE)	All but PE sample Ref 177 (0.159 TEQ) had significantly higher total TEQ than blank. Ref 177 was confirmed by running in another batch and results, which agreed within 18%. Additionally, Ref 177 was compared to its replicates within the program and considered acceptable.
D/F WG12737	N	0.348	25.7–192 (Midland) 35.2– 1,300 (Titta. River soil)	Sample concentrations >20x blank.
D/F WG12804	N	0.0153	3.89–188 (PE)	A few analytes higher than criteria but no significant contribution to total TEQ.
D/F WG13547	N	0.0553	57.5– 3,000 (Nitro) 37.9 (North Carolina) 122 (Saginaw River) 26.4–222 (Midland)	Several analytes exceeded criteria, but blank total TEQ contribution to sample is relatively small.

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
D/F WG13548	N	0.0114	99.6–99.7 (Saginaw River) 32.9–36.4 (North Carolina) 0.268–100 (Extracts)	Several analytes exceeded criteria. In general, the blank contribution to total TEQ was negligible and in those cases results were accepted. Several low-level extract samples were evaluated as follows: Extract Spike #1 samples L6754-4 (Ref 4), -8 (Ref 8), -10 (Ref 10), -14 (Ref 14), -19 (Ref 19), -22 (Ref 22), and -23 (Ref 23) were known TCDD spikes at 0.5 pg/mL. Results were compared to the known spiked TEQ and considered unaffected by blank contribution to TEQ. Extract Spike #3 samples L6754-1 (Ref 1), -7 (Ref 7), -12 (Ref 12), and -15 (Ref 15) were PCB spikes and not expected to contain D/F. These spikes consistently contained a D/F TEQ of ~0.3. However, this came from a consistent ~0.3 pg/mL of TCDD detected in these extracts that was confirmed as a low-level TCDD contamination by AXYS. Since TCDD was not present in the lab blank, these results were accepted as unaffected by any blank contribution to TEQ.
D/F WG13549	N	0.0925	2,160–3,080 (Nitro) 146–1,320 (Saginaw River) 788–8,410 (North Carolina)	Many analytes exceeded limits, but the blank contribution to total TEQ is small relative to sample TEQs.
D/F WG13551	N	2.40	1,100–10,800 (North Carolina) 7,160–11,300 (Winona Post)	Many analytes exceeded limits, but the blank contribution to total TEQ is small relative to sample TEQs.
D/F WG13552	Y	0.000969	0.0386–9.28 (PE) 25.8 (Midland)	
D/F WG13984	N	0.0154	0.524–24.8 (PE) 10.4 (Raritan Bay) 53.1–444 (Extracts)	Blank contribution to total TEQ was negligible except for PE samples L7179-7 (Ref 94), -8 (Ref 96), -11 (Ref 108), -12 (Ref 109), -17 (Ref 132), and L7182-6 (Ref 150). All but L7179-8 were certified blanks. L7179-8 was a PAH spike with no D/F TEQ expected. The TEQs of these samples were considered sufficiently low enough to still be distinguished as blank samples and were accepted.

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
D/F WG14274	N	0.0434	2,800 (Nitro) 35.5–8,320 (North Carolina) 0.0530–5.93 (PE)	Sample TEQs were large enough to be unaffected by the blank TEQ except for four PE samples L7179-4 (Ref 85), -16 (Ref 124) and L7182-12 (Ref 169) and -14 (Ref 184). These PE samples were either certified blanks or PCB spikes with no expected D/F TEQ. Resulting TEQs for these samples were considered low enough to be distinguished as blank samples and were accepted.
PCB WG12108	N	0.000137	2.63–5.19 (Newark Bay) 2.04–2.82 (Raritan Bay)	PCB 77 slightly high, but all samples >20x blank levels.
PCB WG12147	Y	0.000	1.21–5.06 (Newark Bay) 0.104–0.330 (Brunswick)	
PCB WG12265	Y	0.0000584	0.132–0.369 (Brunswick) 0.034–0.649 (Titta. River sediment) 0.00277–1,030 (PE)	
PCB WG12457	N	0.000208	4.20–1,020 (PE)	PCB 77 slightly high. Did not report any samples where PCB 77 was <10x blank. No significant effect on total TEQ.
PCB WG12687	N	0.0183	0.974–2.73 (Midland) 10.3–1,180 (PE)	PCB 77 and 156 high, but all samples >20x blank levels.
PCB WG12834	N	0.000405	0.0157–62.4 (Saginaw River) 0.181–0.203 (Brunswick) 0.986–7.57 (Titta. River Soil)	PCB 77 slightly high. Does not affect total TEQ.
PCB WG12835	N	0.000125	0.822–2.06 (Winona Post)	PCB 77 slightly high. Sample TEQs much greater than blank TEQ.
PCB WG12836	N	0.0499	1060–904,000 (North Carolina)	PCBs 77, 123, 126, 156, 167, and 118 high, but most samples significantly > 20x blank levels
PCB WG13008	N	0.0221	2.38–3.15 (Midland) 1.03–8.37 (Titta. River soil) 41.0–1140 (PE)	PCBs 77 and 118 high, but all samples >20x blank levels.
PCB WG13256	Y	0.000102	0.00385–0.051 (PE)	
PCB WG13257	Y	0.000251	0.253–0.318 (Midland) 0.135–2.08 (Extracts) 3.53–9.62 (PE) 1.14–1.33 (Titta. River Soil)	

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
PCB WG13258	Y	0.000301	0.163–37.0 (Nitro) 29.8–73.6 (Saginaw River) 40.1–42.1 (PE)	
PCB WG13554	N	0.0000900	0.000103–1,080 (Extracts) 435–1,160 (PE)	PCB 77 slightly high. Does not affect total TEQ.
PCB WG14109	N	0.000288	0.388–0.452 (Nitro) 0.0467 (Saginaw River) 0.654–1.87 (Winona Post) 0.00300–0.0420 (PE)	PCB 77 high. PE certified blanks Ref 85, Ref 85 duplicate, and Ref 108 were the only samples where PCB 77 was not >20x blank. TEQs for these certified blanks were considered low enough to be distinguished as blank samples and were accepted.

[a] All nondetect and EMPC values were assigned a zero concentration for the TEQ calculation.
[b] "Ref XX" is a reference laboratory sample ID number.

Table C-2. Sample Batch Duplicate Summary

Sample Batch Number	Criteria Met	Duplicate RPD[a] (%)	Comments
D/F WG12107	N	23	L6744-5, Ref 100 Newark Bay Because this was above the 20% criteria, an additional aliquot of this sample was prepared. Results for the additional aliquot were within 11% RPD from the original results; therefore, this duplicate result was accepted.
D/F WG12148	Y	2.1	L6744-9, Ref 122 Newark Bay
D/F WG12264	Y	1.2	L6760-2, Ref 27 PE
D/F WG12534	Y	5.7	L6760-14, Ref 55 PE
D/F WG12641	Y	4.6	L6747-1, Ref 32 Midland
D/F WG12737	Y	14	L6750-3, Ref 78 Tittabawassee River Soil
D/F WG12804	N	none	The duplicate processed with this batch was to be repeated due to some analytes being <20x blank level. However, it was reprocessed as a single sample and not a duplicate. Samples in this set were accepted based on their agreement with other replicates within the demonstration program.
D/F WG13547	Y	16	L7163-1, Ref 26 Nitro
D/F WG13548	Y	5.9	L6751-14, Ref 83 North Carolina
D/F WG13549	Y	3.6	L6751-7, Ref 135 North Carolina
D/F WG13551	Y	0.0	L6751-1, Ref 42 North Carolina
D/F WG13552	Y	20 (on U=1/2 DL basis[b])	L7179-3, Ref 74 PE. Fails on a U=0 DL basis due to presence of "K" flagged analytes in one replicate. When compared on U-1/2 DL basis where "K" concentrations are included in the TEQ calculation, the duplicate passed.
D/F WG13984	Y	3.4	L7179-14, Ref 113 PE
D/F WG14274	N	54	L7179-16, Ref 124 PE This was a PCB PE sample and contained only trace levels of D/F. Replicate precision is affected because D/F content is so low. This is not expected to indicate any problems with precision within this sample set. Samples in this set were accepted based on their agreement with other replicates within the demonstration program.

Sample Batch Number	Criteria Met	Duplicate RPD[a] (%)	Comments
PCB WG12108	N	22	L6744-2, Ref 49 Newark Bay This result is only slightly above the acceptance criteria of 20%. The variability was influenced by 25% RPD for PCB126 (which has the highest TEF of the PCBs and, therefore, a larger influence on total TEQ). The slight exceedance in duplicate criteria was not considered to have any significant impact on the data reported in this sample batch. All samples in this set were also evaluated based on their agreement with other replicates within the demonstration program and deemed to be acceptable.
PCB WG12147	N	none	L6748-9, Ref 129 Brunswick The duplicate sample for this batch required reprocessing. When reprocessed, it was not prepared in duplicate. Samples in this set were accepted based on the RPD of site replicates that were processed within the batch (RPDs <10%).
PCB WG12265	Y	2.5	L6760-5, Ref 35 PE
PCB WG12457	N	none	L6760-16, Ref 62 PE This duplicate set was to be repeated due to low internal standard recovery. When repeated, it was not prepared in duplicate. Data for this set was accepted because all samples in the set were PE samples. These PE samples met accuracy criteria and reproducibility criteria to other replicates of the same PE material processed within the demonstration.
PCB WG12687	Y	4.3	L6762-12, Ref 169 PE
PCB WG12834	Y	4.2	L6750-8, Ref 164 Tittabawassee River Soil
PCB WG12835	N	none	Duplicate sample repeated in WG13258. Results reported with that sample set. Three sets of sample replicates within this batch were also compared and found to have <13.5% RPD showing acceptable precision with this sample set.
PCB WG12836	Y	2.6	L6751-6, Ref 126 North Carolina
PCB WG13008	Y	5.1	L6750-6, Ref 121 Tittabawassee River Soil

Sample Batch Number	Criteria Met	Duplicate RPD[a] (%)	Comments
PCB WG13256	Y	1.7 (on U=1/2 DL basis)	L6761-3, Ref 74 PE. Fails on a U=0 DL basis due to presence of "K" flagged analytes in one replicate. When compared on U=1/2 DL basis where "K" concentrations are included in the TEQ calculation, the duplicate passed.
PCB WG13257	Y	15	L7187-5, Ref 92 Tittabawassee River Soil
PCB WG13258	Y	19	L6743-2, Ref 36 Nitro
PCB WG13554	Y	12	L6762-1, Ref 202 PE
PCB WG14109	N	85 (on U=1/2 DL basis)	L7179-4, PE. Fails based on both U=0 and U=1/2 DL. This was a blank PE sample and contained only trace levels of PCBs. Replicate precision is affected because the PCB content is so low. This is not expected to indicate any problems with precision within this sample set. Samples in this set were accepted based on their agreement with other replicates within the demonstration program.

[a] Nondetects were assigned a concentration of zero unless otherwise noted and are referred to as U=0 DL values.
[b] U=1/2 DL indicates that nondetects were assigned a concentration equal to one-half the SDL and EMPC concentrations were assigned a value equal to the EMPC.

Appendix D
Summary of Developer and Reference Laboratory Data

Appendix D. XDS and Reference Laboratory One-to-One Matching

Sample Type	Sample Number	Measurement Location	Sample Description	REP	TEQ_{PCB} (pg/g)		$TEQ_{D/F}$ (pg/g)		Total TEQ (pg/g) [c]	
					Developer[a]	Reference Laboratory[b]	Developer[a]	Reference Laboratory[b]	Developer	Reference Laboratory
Environmental	XDS 30	Field	Brunswick #1	1	NR[d]	0.314	678.4	67.2	678.40	67.51
Environmental	XDS 180	Laboratory	Brunswick #1	2	37.50	0.342	1781.95	71.6	1819.45	71.94
Environmental	XDS 148	Laboratory	Brunswick #1	3	ND<0.88 (187.95)[e]	0.369	391.18	61.7	391.18	62.07
Environmental	XDS 66	Laboratory	Brunswick #1	4	2.31	0.313	383.57	67.8	385.88	68.11
Environmental	XDS 202	Laboratory	Brunswick #2	1	187.95 (ND<0.88)[e]	0.127	713.45	49.5	901.40	49.63
Environmental	XDS 168	Laboratory	Brunswick #2	2	ND<0.88	0.128	675.77	72.8	675.77	72.93
Environmental	XDS 46	Laboratory	Brunswick #2	3	ND<0.63	0.132	370.87	56	370.87	56.13
Environmental	XDS 70	Laboratory	Brunswick #2	4	2.60	0.123	390.37	60.4	392.97	60.52
Environmental	XDS 48	Laboratory	Brunswick #3	1	ND<0.63	0.19	58756.79	12600	58756.79	12600.19
Environmental	XDS 150	Laboratory	Brunswick #3	2	176.26	0.181	282954.07	15200	283130.33	15200.18
Environmental	XDS 121	Laboratory	Brunswick #3	3	1710.37	0.203	327462.3	13100	329172.67	13100.20
Environmental	XDS 120	Laboratory	Brunswick #3	4	2702.31	0.182	341129.02	13600	343831.33	13600.18
Environmental	XDS 76	Laboratory	Midland #1	1	ND<0.63	2.59	652.4	222	652.40	224.59
Environmental	XDS 169	Laboratory	Midland #1	2	58.26	2.73	811.10	241	869.36	243.73
Environmental	XDS 209	Laboratory	Midland #1	3	106.33	2.5	1018.37	269	1124.70	271.50
Environmental	XDS 79	Laboratory	Midland #1	4	0.87	2.53	734.47	268	735.34	270.53
Environmental	XDS 201	Laboratory	Midland #2	1	7.62	2.7	837.69	208	845.31	210.70
Environmental	XDS 137	Laboratory	Midland #2	2	ND<0.88	2.81	456.94	179	456.94	181.81
Environmental	XDS 207	Laboratory	Midland #2	3	ND<0.88	2.48	744.96	197	744.96	199.48
Environmental	XDS 164	Laboratory	Midland #2	4	ND<0.88	3.15	935.82	192	935.82	195.15
Environmental	XDS 122	Laboratory	Midland #3	1	ND<0.63	2.28	558.19	185	558.19	187.28
Environmental	XDS 40	Field	Midland #3	2	2.58	2.17	568.95	174	571.53	176.17
Environmental	XDS 103	Laboratory	Midland #3	3	1.62	2.23	500.23	176	501.85	178.23
Environmental	XDS 186	Laboratory	Midland #3	4	13.78	2.38	934.75	161	948.53	163.38
Environmental	XDS 60	Laboratory	Midland #4	1	26.63	0.253	35.43	25.7	62.06	25.95
Environmental	XDS 126	Laboratory	Midland #4	2	ND<0.42	0.318	43.23	26.4	43.23	26.72
Environmental	XDS 161	Laboratory	Midland #4	3	ND<0.42	0.974	37.58	31	37.58	31.97
Environmental	XDS 63	Laboratory	Midland #4	4	1.03	0.263	57.74	25.8	58.77	26.06
Environmental	XDS 185	Laboratory	NC PCB Site #1	1	9611.90	53000	32412.81	788	42024.71	53788.00
Environmental	XDS 133	Laboratory	NC PCB Site #1	2	13487.00	65300	25719.60	1100	39206.60	66400.00
Environmental	XDS 127	Laboratory	NC PCB Site #1	3	28334.32	80500	26370.35	852	54704.67	81352.00

Sample Type	Sample Number	Measurement Location	Sample Description	REP	TEQ_{PCB} (pg/g)		$TEQ_{D/F}$ (pg/g)		Total TEQ (pg/g) [c]	
					Developer[a]	Reference Laboratory[b]	Developer[a]	Reference Laboratory[b]	Developer	Reference Laboratory
Environmental	XDS 90	Laboratory	NC PCB Site #1	4	30376.54	85100	28586.20	906	58962.74	86006.00
Environmental	XDS 160	Laboratory	NC PCB Site #2	1	81581.94	311000	801924.49	3400	883506.43	314400.00
Environmental	XDS 172	Laboratory	NC PCB Site #2	2	77272.76	305000	698405.79	3300	775678.55	308300.00
Environmental	XDS 181	Laboratory	NC PCB Site #2	3	89977.32	210000	707390.20	3430	797367.52	213430.00
Environmental	XDS 35	Laboratory	NC PCB Site #2	4	>39302.35	361000	>47853.33	3490	87155.68	364490.00
Environmental	XDS 128	Laboratory	NC PCB Site #3	1	156638.77	848000	631785.36	8320	788424.13	856320.00
Environmental	XDS 187	Laboratory	NC PCB Site #3	2	94523.48	618000	465880.06	8410	560403.54	626410.00
Environmental	XDS 197	Laboratory	NC PCB Site #3	3	95840.47	533000	416820.65	9360	512661.12	542360.00
Environmental	XDS 117	Laboratory	NC PCB Site #3	4	79055.94	904000	677660.44	10800	756716.38	914800.00
Environmental	XDS 88	Laboratory	Newark Bay #1	1	ND<0.50	1.22	17.43	23	17.43	24.22
Environmental	XDS 141	Laboratory	Newark Bay #1	2	ND<0.42	1.44	33.76	14	33.76	15.44
Environmental	XDS 112	Laboratory	Newark Bay #1	3	2.98	1.39	28.80	14.5	31.78	15.89
Environmental	XDS 154	Laboratory	Newark Bay #1	4	ND<0.42	1.34	40.35	13.5	40.35	14.84
Environmental	XDS 93	Laboratory	Newark Bay #2	1	1.31	5.01	92.25	50.6	93.56	55.61
Environmental	XDS 132	Laboratory	Newark Bay #2	2	ND<0.42	5.19	256.22	47.4	256.22	52.59
Environmental	XDS 77	Laboratory	Newark Bay #2	3	ND<0.50	5.14	83.38	74.1	83.38	79.24
Environmental	XDS 50	Laboratory	Newark Bay #2	4	6.39	5.09	98.99	50.4	105.38	55.49
Environmental	XDS 191	Laboratory	Newark Bay #3	1	ND<0.42	4.61	48.12	38.9	48.12	43.51
Environmental	XDS 75	Laboratory	Newark Bay #3	2	ND<0.50	5.04	59.28	44.9	59.28	49.94
Environmental	XDS 182	Laboratory	Newark Bay #3	3	ND<0.42	4.5	40.83	40.2	40.83	44.70
Environmental	XDS 64	Laboratory	Newark Bay #3	4	ND<0.5	5.03	90.68	41.9	90.68	46.93
Environmental	XDS 29	Field	Newark Bay #4	1	NR	2.73	83.4	33.6	83.40	36.33
Environmental	XDS 146	Laboratory	Newark Bay #4	2	ND<0.42	2.65	54.83	26.1	54.83	28.75
Environmental	XDS 156	Laboratory	Newark Bay #4	3	ND<0.42	2.72	39.47	27.6	39.47	30.32
Environmental	XDS 178	Laboratory	Newark Bay #4	4	ND<0.42	2.7	23.79	26.8	23.79	29.50
Environmental	XDS 193	Laboratory	Raritan Bay #1	1	ND<0.42	2.33	23.42	10.2	23.42	12.53
Environmental	XDS 176	Laboratory	Raritan Bay #1	2	ND<0.42	2.06	29.64	10.3	29.64	12.36
Environmental	XDS 131	Laboratory	Raritan Bay #1	3	ND<0.42	2.35	19.04	10.4	19.04	12.75
Environmental	XDS 56	Laboratory	Raritan Bay #1	4	ND<0.50	2.25	23.75	11.4	23.75	13.65
Environmental	XDS 53	Laboratory	Raritan Bay #2	1	7.25	2.7	22.54	13.3	29.79	16.00
Environmental	XDS 174	Laboratory	Raritan Bay #2	2	ND<0.42	2.67	29.05	13.1	29.05	15.77
Environmental	XDS 205	Laboratory	Raritan Bay #2	3	ND<0.75	2.68	31.29	12.8	31.29	15.48
Environmental	XDS 80	Laboratory	Raritan Bay #2	4	ND<1.90	2.85	38.20	13	38.20	15.85
Environmental	XDS 167	Laboratory	Raritan Bay #3	1	ND<0.42	2.43	25.92	10.4	25.92	12.83
Environmental	XDS 67	Laboratory	Laboratory Raritan Bay #3	2	ND<0.50	2.43	23.74	11.1	23.74	13.53

Sample Type	Sample Number	Measurement Location	Sample Description	REP	TEQ_{PCB} (pg/g)		$TEQ_{D/F}$ (pg/g)		Total TEQ (pg/g) [c]	
					Developer[a]	Reference Laboratory[b]	Developer[a]	Reference Laboratory[b]	Developer	Reference Laboratory
Environmental	XDS 165	Laboratory	Raritan Bay #3	3	ND<0.42	2.3	37.32	10.6	37.32	12.90
Environmental	XDS 190	Laboratory	Raritan Bay #3	4	ND<0.42	2.33	24.58	9.93	24.58	12.26
Environmental	XDS 82	Laboratory	Saginaw River #1	1	4.20	62.4	2940.06	1050	2944.26	1112.40
Environmental	XDS 55	Laboratory	Saginaw River #1	2	12.43	73.6	3189.20	683	3201.63	756.60
Environmental	XDS 44	Laboratory	Saginaw River #1	3	9.77	69.9	4091.26	1070	4101.03	1139.90
Environmental	XDS 39	Field	Saginaw River #1	4	117.35	63.7	2340	694	2457.35	757.70
Environmental	XDS 124	Laboratory	Saginaw River #2	1	13.76	30.6	2729.40	1110	2743.16	1140.60
Environmental	XDS 84	Laboratory	Saginaw River #2	2	11.92	31	5209.46	953	5221.38	984.00
Environmental	XDS 159	Laboratory	Saginaw River #2	3	121.67	26.7	4096.12	1320	4217.79	1346.70
Environmental	XDS 104	Laboratory	Saginaw River #2	4	5.77	29.8	2838.39	864	2844.16	893.80
Environmental	XDS 25	Field	Saginaw River #3	1	NR	0.0202	551.27	99.7	551.27	99.72
Environmental	XDS 188	Laboratory	Saginaw River #3	2	1.55	0.0164	575.77	146	577.32	146.02
Environmental	XDS 95	Laboratory	Saginaw River #3	3	ND<0.50	0.0467	555.02	122	555.02	122.05
Environmental	XDS 118	Laboratory	Saginaw River #3	4	ND<0.50	0.0157	578.03	99.6	578.03	99.62
Environmental	XDS 109	Laboratory	Solutia #1	1	ND<0.63	0.452	210.27	57.5	210.27	57.95
Environmental	XDS 91	Laboratory	Solutia #1	2	ND<0.63	0.163	191.53	76.9	191.53	77.06
Environmental	XDS 42	Field	Solutia #1	3	2.44	0.388	293.15	62	295.59	62.39
Environmental	XDS 52	Laboratory	Solutia #1	4	3.11	0.391	177.54	61.6	180.65	61.99
Environmental	XDS 86	Laboratory	Solutia #2	1	2.57	17.6	2310.65	2090	2313.22	2107.60
Environmental	XDS 199	Laboratory	Solutia #2	2	4.24	18.8	2063.31	1950	2067.55	1968.80
Environmental	XDS 115	Laboratory	Solutia #2	3	1.86	19.2	4730.74	1860	4732.60	1879.20
Environmental	XDS 136	Laboratory	Solutia #2	4	56.53	18.5	23.96	2160	80.49	2178.50
Environmental	XDS 98	Laboratory	Solutia #3	1	4.21(NR)[e]	29.7	2930.80	2810	2935.01	2839.70
Environmental	XDS 177	Laboratory	Solutia #3	2	1820.53	36.9	7621.37	2800	9441.90	2836.90
Environmental	XDS 200	Laboratory	Solutia #3	3	38.16	37	2122.88	3000	2161.04	3037.00
Environmental	XDS 142	Laboratory	Solutia #3	4	32.98	31.5	6480.03	3080	6513.01	3111.50
Environmental	XDS 119	Laboratory	Titta. River Soil #1	1	0.76	7.32	225.88	35	226.64	42.32
Environmental	XDS 69	Laboratory	Titta. River Soil #1	2	0.80	8.26	132.25	35.2	133.05	43.46
Environmental	XDS 140	Laboratory	Titta. River Soil #1	3	1.84	7.57	88.89	40	90.73	47.57
Environmental	XDS 71	Laboratory	Titta. River Soil #1	4	4.15	8.37	97.65	35.8	101.80	44.17
Environmental	XDS 34	Field	Titta. River Soil #2	1	819.39	0.986	1668.25	420	2487.64	420.99
Environmental	XDS 143	Laboratory	Titta. River Soil #2	2	10.01	1.2	1587.89	450	1597.90	451.20
Environmental	XDS 107	Laboratory	Titta. River Soil #2	3	2.46	1.03	1424.72	523	1427.18	524.03
Environmental	XDS 173	Laboratory	Titta. River Soil #2	4	6.34	1.06	2074.71	506	2081.05	507.06
Environmental	XDS 183	Laboratory	Titta. River Soil #3	1	9.83	1.26	3132.01	1050	3141.84	1051.26

Sample Type	Sample Number	Measurement Location	Sample Description	REP	TEQ_{PCB} (pg/g)		$TEQ_{D/F}$ (pg/g)		Total TEQ (pg/g) [c]	
					Developer[a]	Reference Laboratory[b]	Developer[a]	Reference Laboratory[b]	Developer	Reference Laboratory
Environmental	XDS 74	Laboratory	Titta. River Soil #3	2	ND<0.50	1.16	1932.38	676	1932.38	677.16
Environmental	XDS 59	Laboratory	Titta. River Soil #3	3	23.44	1.54	16722.28 (3353.51)[e]	1220	16745.72	1221.54
Environmental	XDS 144	Laboratory	Titta. River Soil #3	4	13.10	1.33	1650.17	1300	1663.27	1301.33
Environmental	XDS 145	Laboratory	Titta. River Sed #1	1	ND<0.42	0.0527	6.90	1.05	6.90	1.10
Environmental	XDS 73	Laboratory	Titta. River Sed #1	2	ND<0.50	0.034	1.88	1.11	1.88	1.14
Environmental	XDS 43	Field	Titta. River Sed #1	3	<0.60	0.0407	4.42	1	4.42	1.04
Environmental	XDS 163	Laboratory	Titta. River Sed #1	4	ND<0.42	0.0403	1.66	1.7	1.66	1.74
Environmental	XDS 101	Laboratory	Titta. River Sed #2	1	ND<0.50	0.649	131.24	52.8	131.24	53.45
Environmental	XDS 54	Laboratory	Titta. River Sed #2	2	6.27	0.71	480.42	123	486.69	123.71
Environmental	XDS 58	Laboratory	Titta. River Sed #2	3	ND<0.50	0.566	647.96	66.1	647.96	66.67
Environmental	XDS 24	Field	Titta. River Sed #2	4	1.91	0.515	303.39	94.1	305.30	94.62
Environmental	XDS 153	Laboratory	Titta. River Sed #3	1	ND<0.42	0.0719	32.37	13	32.37	13.07
Environmental	XDS 203	Laboratory	Titta. River Sed #3	2	ND<0.42	0.0973	24.56	11.2	24.56	11.30
Environmental	XDS 139	Laboratory	Titta. River Sed #3	3	ND<0.42	0.083	49.49	12.7	49.49	12.78
Environmental	XDS 196	Laboratory	Titta. River Sed #3	4	ND<0.42	0.09	19.60	13.8	19.60	13.89
Environmental	XDS 130	Laboratory	Winona Post #1	1	186.90	0.654	30696.45	7290	30883.35	7290.65
Environmental	XDS 171	Laboratory	Winona Post #1	2	51.43	0.904	37880.91	7370	37932.34	7370.90
Environmental	XDS 89	Laboratory	Winona Post #1	3	ND<0.63	0.829	34205.98	7450	34205.98	7450.83
Environmental	XDS 97	Laboratory	Winona Post #1	4	ND<0.63	0.822	11048.42 (28400.57)[e]	7160	11048.42	7160.82
Environmental	XDS 110	Laboratory	Winona Post #2	1	2133.92	1.2	252424.73	9720	254558.65	9721.20
Environmental	XDS 198	Laboratory	Winona Post #2	2	21.33	1.3	61670.05	9770	61691.38	9771.30
Environmental	XDS 123	Laboratory	Winona Post #2	3	2149.30	1.32	286476.09	9200	288625.39	9201.32
Environmental	XDS 152	Laboratory	Winona Post #2	4	12.94	1.28	34424.23 (43807.75)[e]	11300	34437.17	11301.28
Environmental	XDS 184	Laboratory	Winona Post #3	1	99.17	1.68	122062.58	10300	122161.75	10301.68
Environmental	XDS 61	Laboratory	Winona Post #3	2	ND<0.63	1.87	50372.99	9770	50372.99	9771.87
Environmental	XDS 41	Field	Winona Post #3	3	172.69	1.8	15502.28	9320	15674.97	9321.80
Environmental	XDS 47	Laboratory	Winona Post #3	4	ND<0.63	2.06	48249.56	9870	48249.56	9872.06
Extract	XDS 22	Field	Envir Extract #1	1	9.18	0.629	489.67	175	498.85	175.63
Extract	XDS 5	Field	Envir Extract #1	2	26.11	0.673	523.29	444	549.40	444.67
Extract	XDS 20	Field	Envir Extract #1	3	9.84	0.64	516.89	176	526.73	176.64
Extract	XDS 15	Field	Envir Extract #1	4	NA	2.08	2304.98	439	2304.98	441.08
Extract	XDS 6	Field	Envir Extract #2	1	0.45	0.742	169.79	55.3	170.24	56.04

Sample Type	Sample Number	Measurement Location	Sample Description	REP	TEQ_{PCB} (pg/g)		$TEQ_{D/F}$ (pg/g)		Total TEQ (pg/g) [c]	
					Developer[a]	Reference Laboratory[b]	Developer[a]	Reference Laboratory[b]	Developer	Reference Laboratory
Extract	XDS 18	Field	Envir Extract #2	2	1.20	0.135	196.45	53.3	197.65	53.44
Extract	XDS 9	Field	Envir Extract #2	3	NR	0.297	193.02	53.1	193.02	53.40
Extract	XDS 16	Field	Envir Extract #2	4	NR	0.17	211.13	53.6	211.13	53.77
Extract	XDS 19	Field	Spike #1	1	<0.07	0.0638	0.43	0.504	0.43	0.57
Extract	XDS 1	Field	Spike #1	2	<0.09	0.00013	<0.13	0.509	NR	0.51
Extract	XDS 7	Field	Spike #1	3	<0.09	0.0001	0.34	0.537	0.34	0.54
Extract	XDS 17	Field	Spike #1	4	<0.07	0.0275	0.24	0.524	0.24	0.55
Extract	XDS 4	Field	Spike #1	5	0.1	0.0562	<0.13	0.585	0.10	0.64
Extract	XDS 12	Field	Spike #1	6	NA	0.00724	0.56	0.576	0.56	0.58
Extract	XDS 3	Field	Spike #1	7	0.12	0.139	<0.13	0.52	0.12	0.66
Extract	XDS 21	Field	Spike #2	1	10.66	113	74.22	91.6	84.88	204.60
Extract	XDS 10	Field	Spike #2	2	NR	113	70.56	91.8	70.56	204.80
Extract	XDS 23	Field	Spike #2	3	12.2	111	69.42	89.1	81.62	200.10
Extract	XDS 14	Field	Spike #2	4	NR	113	98.64	100	98.64	213.00
Extract	XDS 8	Field	Spike #3	1	225.24	1060	97.57	0.324	322.81	1060.32
Extract	XDS 2	Field	Spike #3	2	211.91	1080	46.27	0.348	258.18	1080.35
Extract	XDS 11	Field	Spike #3	3	NR	1060	95.06	0.363	95.06	1060.36
Extract	XDS 13	Field	Spike #3	4	NR	990	58.67	0.268	58.67	990.27
Performance	XDS 195	Laboratory	Cambridge 5183	1	ND<0.42	3.81	26.69	4.78	26.69	8.59
Performance	XDS 113	Laboratory	Cambridge 5183	2	339.41	4.33	26.94	4.08	366.35	8.41
Performance	XDS 72	Laboratory	Cambridge 5183	3	19.18	4.2	14.22	4.06	33.40	8.26
Performance	XDS 38	Field	Cambridge 5183	4	3.23	4.24	28.47	3.56	31.70	7.80
Performance	XDS 108	Laboratory	Cambridge 5183	5	ND<0.5	4.25	22.91	3.89	22.91	8.14
Performance	XDS 87	Laboratory	Cambridge 5183	6	6.70	3.86	29.80	5.93	36.50	9.79
Performance	XDS 111	Laboratory	Cambridge 5183	7	4.63	3.53	18.58	3.89	23.21	7.42
Performance	XDS 155	Laboratory	Cambridge 5184	1	81.22	1080	1033.74	187	1114.96	1267.00
Performance	XDS 114	Laboratory	Cambridge 5184	2	2.74	1120	716.75	188	719.49	1308.00
Performance	XDS 192	Laboratory	Cambridge 5184	3	0.79	1140	1117.68	173	1118.47	1313.00
Performance	XDS 31	Field	Cambridge 5184	4	NR	1160	807.46	180	807.46	1340.00
Performance	XDS 37	Field	ERA Aroclor	1	1690.23	1060	170.37	36.4	1860.60	1096.40
Performance	XDS 138	Laboratory	ERA Aroclor	2	110.72	3690	297.76	32.9	408.48	3722.90
Performance	XDS 28	Field	ERA Aroclor	3	NR	3790	167.41	37.9	167.41	3827.90
Performance	XDS 189	Laboratory	ERA Aroclor	4	ND<0.42 (69.77)[e]	3800	175.10	35.5	175.10	3835.50
Performance	XDS 106	Laboratory	ERA Blank	1	13.72	0.0243	ND<0.45	0.0942	13.72	0.12

Sample Type	Sample Number	Measurement Location	Sample Description	REP	TEQ_{PCB} (pg/g)		$TEQ_{D/F}$ (pg/g)		Total TEQ (pg/g) [c]	
					Developer[a]	Reference Laboratory[b]	Developer[a]	Reference Laboratory[b]	Developer	Reference Laboratory
Performance	XDS 116	Laboratory	ERA Blank	2	0.88	0.00385	ND<0.23	0.0728	0.88	0.08
Performance	XDS 92	Laboratory	ERA Blank	3	1.02	0.00277	ND<0.45	0.237	1.02	0.24
Performance	XDS 51	Laboratory	ERA Blank	4	6.87	0.042	ND<0.45	0.307	6.87	0.35
Performance	XDS 105	Laboratory	ERA Blank	5	ND<1.26	0.0229	ND<0.45	0.113	NA	0.14
Performance	XDS 81	Laboratory	ERA Blank	6	1.91	0.0191	ND<0.45	0.0524	1.91	0.07
Performance	XDS 100	Laboratory	ERA Blank	7	ND<0.50	0.0325	0.75	0.211	0.75	0.24
Performance	XDS 32	Field	ERA Blank	8	5.67	0.0225	13.74	0.0692	19.41	0.09
Performance	XDS 147	Laboratory	ERA PAH	1	ND<0.42	0.0254	ND<0.45	0.159	NR	0.18
Performance	XDS 135	Laboratory	ERA PAH	2	ND<0.42	0.00429	1.16	0.141	1.16	0.15
Performance	XDS 96	Laboratory	ERA PAH	3	27.34	0.00423	ND<0.45	0.161	27.34	0.17
Performance	XDS 27	Field	ERA PAH	4	NR	0.026	2.92	0.248	2.92	0.27
Performance	XDS 166	Laboratory	ERA PCB 100	1	ND<0.42	10.6	0.46	0.0386	0.46	10.64
Performance	XDS 94	Laboratory	ERA PCB 100	2	1.88	11.1	ND<0.45	NA[f]	1.88	NA[f]
Performance	XDS 83	Laboratory	ERA PCB 100	3	14.77	10.6	0.75	0.053	15.52	10.65
Performance	XDS 125	Laboratory	ERA PCB 100	4	ND<0.42	9.95	1.99	0.127	1.99	10.08
Performance	XDS 204	Laboratory	ERA PCB 10000	1	51.17	1030	252.75	0.204	303.92	1030.20
Performance	XDS 68	Laboratory	ERA PCB 10000	2	26.31	1030	206.93	0.507	233.24	1030.51
Performance	XDS 134	Laboratory	ERA PCB 10000	3	46.47	1180	19.60	0.105	66.07	1180.11
Performance	XDS 162	Laboratory	ERA PCB 10000	4	31.27	1020	21.62	0.0628	52.89	1020.06
Performance	XDS 45	Laboratory	ERA TCDD 10	1	5.35	0.0147	10.78	8.69	16.13	8.70
Performance	XDS 149	Laboratory	ERA TCDD 10	2	ND<0.42	0.0123	22.81	9.28	22.81	9.29
Performance	XDS 175	Laboratory	ERA TCDD 10	3	ND<0.42	0.0299	14.67	8.44	14.67	8.47
Performance	XDS 158	Laboratory	ERA TCDD 10	4	ND<0.42	0.045	16.83	8.2	16.83	8.25
Performance	XDS 36	Field	ERA TCDD 30	1	1.86	0.0451	45.66	27.4	47.52	27.45
Performance	XDS 129	Laboratory	ERA TCDD 30	2	ND<0.42	0.0153	44.46	25.3	44.46	25.32
Performance	XDS 85	Laboratory	ERA TCDD 30	3	ND<0.5	0.0436	32.77	24.8	32.77	24.84
Performance	XDS 151	Laboratory	ERA TCDD 30	4	ND<0.42	0.04	35.42	23.9	35.42	23.94
Performance	XDS 78	Laboratory	LCG CRM-529	1	211.50	435	15243.48	NA[f]	15454.98	NA[f]
Performance	XDS 65	Laboratory	LCG CRM-529	2	142.50	405	15448.41	6930	15590.91	7335.00
Performance	XDS 62	Laboratory	LCG CRM-529	3	135.27	498	16448.80	6900	16584.07	7398.00
Performance	XDS 26	Field	LCG CRM-529	4	NR	356	15684.8	7190	15684.80	7546.00
Performance	XDS 57	Laboratory	NIST 1944	1	8.27	40.1	885.45	237	893.72	277.10
Performance	XDS 157	Laboratory	NIST 1944	2	ND<0.88	43.7	591.64	206	591.64	249.70
Performance	XDS 179	Laboratory	NIST 1944	3	4.01	42.1	776.07	252	780.08	294.10
Performance	XDS 102	Laboratory	NIST 1944	4	2.01	41	573.89	219	575.90	260.00

Sample Type	Sample Number	Measurement Location	Sample Description	REP	TEQ_{PCB} (pg/g)		$TEQ_{D/F}$ (pg/g)		Total TEQ (pg/g) [c]	
					Developer[a]	Reference Laboratory[b]	Developer[a]	Reference Laboratory[b]	Developer	Reference Laboratory
Performance	XDS 206	Laboratory	Wellington WMS-01	1	ND<0.75	10.6	201.61	68	201.61	78.60
Performance	XDS 49	Laboratory	Wellington WMS-01	2	9.21	9.4	177.64	65.7	186.85	75.10
Performance	XDS 194	Laboratory	Wellington WMS-01	3	ND<0.42	9.62	203.56	61.9	203.56	71.52
Performance	XDS 99	Laboratory	Wellington WMS-01	4	13.22	9.07	138.57	66.1	151.79	75.17
Performance	XDS 170	Laboratory	Wellington WMS-01	5	ND<0.42	10.3	290.81	68	290.81	78.30
Performance	XDS 208	Laboratory	Wellington WMS-01	6	ND<0.75	9.62	201.83	65.7	201.83	75.32
Performance	XDS 33	Field	Wellington WMS-01	7	524.54	9.68	228.59	65.4	753.13	75.08

[a] Data listed exactly as reported by the developer.

[b] Qualifier flags (e.g., J and K flags) included in the raw data have been removed for the purposes of statistical analysis.

[c] Data calculated by summing TEQ_{PCB} and $TEQ_{D/F}$.

[d] NR = result not available.

[e] Revised result provided by XDS after demonstration period. Original result was used in the data analysis.

[f] Reference laboratory data was discarded due to laboratory sample preparation error.